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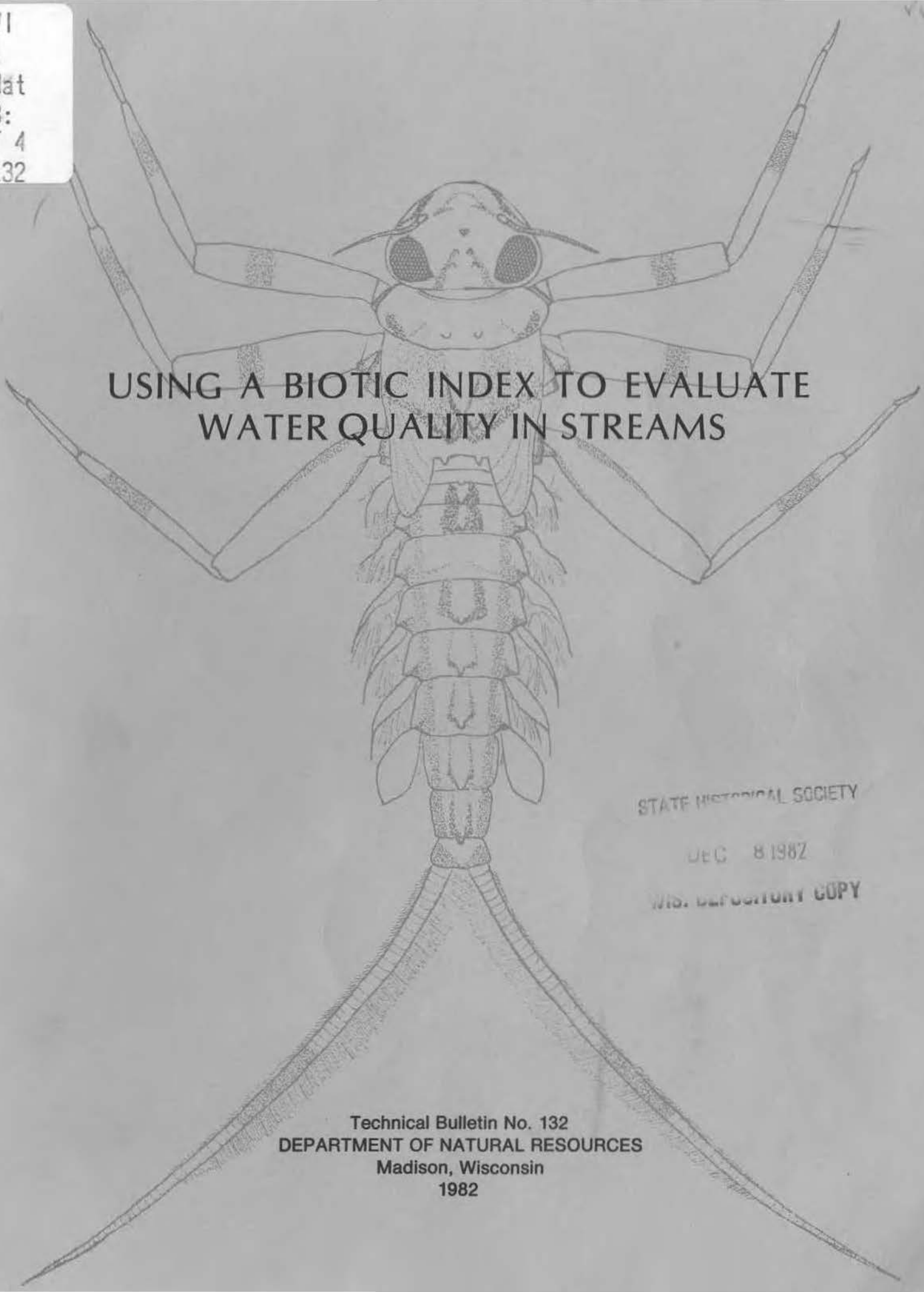

USING A BIOTIC INDEX TO EVALUATE WATER QUALITY IN STREAMS

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By
William L. Hilsenhoff

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INTRODUCTION

The biotic index I proposed in 1977 has been widely used in Wisconsin and elsewhere to evaluate the water quality of streams. It has proven to be a valuable tool, but it is not yet perfected and results obtained through its use must be evaluated with caution. In the past two years we have used the index to evaluate more than 1,000 streams in Wisconsin and have improved our understanding of its use. We have carried out studies to determine the efficiency and accuracy of the index, have evaluated alternative sampling techniques, and have made substantial changes in many of the tolerance values. In this bulletin I wish to report on recent improvements in the biotic index, point out problems that need to be considered when evaluating results, and provide keys for identification of species in certain important insect genera.

DEVELOPMENT OF THE BIOTIC INDEX

Since the primary effect of water pollution is on living organisms, assessment of water quality is principally a biological problem. Biological assessment of water quality has been discussed by Hynes (1960), Cairns and Dickson (1973), and many others, and aquatic macroinvertebrates have proven especially valuable for this purpose (Chandler 1970, Gaufin 1973, Roback 1974). To aid in the interpretation of data, indexes have been developed. Diversity indexes have received wide attention (Wilhm 1970, Zand 1976, Hughes 1978), but are not reliable in most situations (Cook 1976, Hilsenhoff 1977, Murphy 1978) and have not been used extensively by aquatic biologists as a tool for measuring water quality. Chandler (1970) proposed a "biotic score", and with modifications by Cook (1976) and others it has proven more reliable than diversity indexes for evaluating water quality (Murphy 1978).

In Europe and the USSR saprobic systems, which evaluate rates of organic decomposition, have been used extensively to monitor water quality, but their use has not been generally accepted in Great Britain and North America. Sladecek (1973) comprehensively reviewed the literature on saprobic systems and their use in measuring water quality. Most proposed saprobic systems involve extensive analysis at the species level of all organisms from bacteria to insects and fish, and while the results may be precise, such a great expenditure of time is probably not warranted when the only objective is evaluation of water quality.

After a two-year study of 53 Wisconsin streams, I proposed using a biotic index of arthropod populations as a rapid method for evaluating water quality (Hilsenhoff 1977). This index is similar to the saprobic index of Pantel and Buck (1955) and the biotic index of Chutter (1971), but uses only insects, amphipods, and isopods. Beck (1955), Howmiller and Scott (1977), and Winget and Mangum (1979) have also proposed biotic indexes that differ somewhat in their details. I use only insects, amphipods, and isopods in my index because they are generally abundant and easily collected from most streams, their fauna is diverse and not mobile, and most species have life cycles of one year or more.

For the purpose of calculating a biotic index, species are assigned pollution tolerance values of 0 to 5 on the basis of previous field studies (Hilsenhoff 1977)—a 0 value is assigned to species found only in unaltered streams of very high water quality, and a value of 5 is assigned to species known to occur in severely polluted or disturbed streams. Intermediate values are assigned to species that occur in streams with intermediate degrees of pollution or disturbance. When species cannot be identified, genera are assigned values instead. The biotic index is calculated from the formula

$$B.I. = \frac{\sum n_i a_i}{N},$$

where n_i is the number of individuals of each species (or genus), a_i is the tolerance value assigned to that species (or genus), and N is the total number of individuals in the sample. The index is an average of tolerance values, and measures saprobity (rate of organic decomposition) and to some extent trophism, which frequently influences saprobity (Caspers and Karbe 1966). In Wisconsin, the introduction of organic matter or nutrients into a stream and effects of dams are the major causes of deterioration of water quality. Resulting increases in saprobity and trophism are readily detected by the biotic index. Heated discharges, heavy metals, and other toxic substances may also be detected by the index, but their effects on the biotic index have not been evaluated. Bacterial and radioactive pollutants must be detected by other means.

The procedure initially recommended for collecting arthropods for evaluation of water quality with the biotic index (Hilsenhoff 1977) is as follows: "Use a D-frame aquatic net to sample riffles by disturbing the substrate above the net and allowing dislodged arthropods to be washed into the net by the current. If riffles are absent, rock or gravel runs or debris may be similarly sampled. Place a sample containing about 100 arthropods in a shallow white pan containing a little water. When collecting the samples it is important to not collect significantly more than 100 arthropods because in large samples, larger and more easily captured arthropods will be most readily removed from the pan, creating a biased sample. Using a curved forceps, remove and preserve in 70% ethanol arthropods still clinging to the net and those in the pan until 100 have been obtained. Do not collect arthropods less than 3 mm long, except adult Elmidae, because they are difficult to sample and identify. If 100 arthropods cannot be found in 30 minutes, those collected within that time period would constitute a sample."

EVALUATION OF COLLECTION PROCEDURE

Beginning in 1977 several studies were carried out to determine the efficiency and reliability of this procedure, the importance of species identification, and the relative merits of alternative sampling and sorting procedures. The results of these studies are reported below.

TIME REQUIRED FOR COLLECTING, SORTING, AND IDENTIFICATION

To learn exactly how long it takes to evaluate the water quality of a stream using the recommended procedure, and to determine if precision gained by species identifications warrants the additional expenditure of time, a study of 53 Wisconsin streams was initiated in 1977. These were the same 53 streams previously studied (Hilsenhoff 1977), and were selected because they encompassed a wide range of sizes, currents, substrates, water chemistries, and water quality.

Materials and Methods

Sampling was initiated May 20 and completed June 8, with streams farthest south being sampled first. A sample was collected from each stream according to procedures already described. Hemiptera and adults of Dytiscidae, Gyrinidae, Haliplidae, and Hydrophilidae were not collected because they do not rely on the stream for oxygen. If 100 arthropods were not obtained in the first sample, an additional sample was collected. If 100 arthropods were not collected in one-half hour, the number collected in that time period was used as the sample.

In the laboratory, samples from the 53 streams were divided at random into two groups. I sorted the arthropods in 26 samples into 1-dram vials, identified them to genus, and labeled the vials. The remaining 27 samples were similarly sorted and labeled by a student with no entomological training and only one week of experience in sorting such samples, but she did not attempt identification. I later identified these specimens to genus and corrected errors in sorting. Numbers in each genus were recorded and a biotic index was calculated for each stream using published values (Hilsenhoff 1977) and values for genera based on weighted averages of species values. I then identified to species insects in the genera listed in Table 1 and calculated a biotic index using tolerance values for these species. The time needed for each laboratory procedure was recorded, as was the time elapsed from arrival of the vehicle at each stream until its departure.

Except for the Arkansaw River and Wisconsin River #4, the 53 stream sites sampled in 1977 were sampled again in 1978 at the same time of the year, some in conjunction with another experiment which is reported below. *Hydropsyche* and some *Symphitopsyche* were identified to species in both the 1977 and 1978 samples, and species biotic index values were calculated for each year using new tolerance values published in this bulletin. Generic biotic index values were also calculated using weighted tolerance values as follows: *Baetis* 2, *Ephemerella* 1, *Eurylophella* 1, *Serratella* 1, *Heptagenia* 2, *Stenonema* 2, *Brachycentrus* 1, *Hydropsyche* 3, *Symphitopsyche* 2, *Chimarra* 2, *Dubiraphia* 3, *Optioservus* 2, *Stenelmis* 3, *Eusimulium* 2, and *Simulium* 3.

TABLE 1. Average time required to perform tasks necessary for calculation of a biotic index and average numbers of arthropods involved.

Task	Minutes/ Sample	Number/ Sample
Collection of Sample	21.4	
Sorting and generic identification (Except Chironomidae)	32.4	95.8
Identification of Chironomidae	4.1	4.7
Enumeration of samples	4.6	
Calculations	1.5	
Totals for calculation of biotic index at generic level	64.0	100.5
Species identification 712 *Baetis*	7.8	13.4
Species identification 259 *Ephemerella*	2.6	4.9
Species identification 113 *Heptagenia* and 170 *Stenonema*	2.8	5.3
Species identification 24 *Chimarra*	0.4	0.5
Species identification 65 *Brachycentrus*	0.9	1.2
Species identification 272 *Stenelmis*	1.7	5.1
Species identification 36 *Dubiraphia* and 151 *Optioservus*	0.9	3.5
Species identification 197 *Simulium* and 4 *Eusimulium*	1.4	3.8
Enumeration of species	2.5	36.8
Totals for calculation of biotic index at species level	85.0	

Results and Discussion

The time required for me to collect a sample, sort it, identify the arthropods to genus, label the vials, and calculate a biotic index was only slightly more than one hour for each stream (Table 1). To calculate a biotic index based on species, only 21 minutes more were needed for identification and enumeration of species in selected genera (Table 1). Species were not identified in genera where all species had the same index value or where species keys did not exist. As species in certain important families such as Hydropsychidae and Caenidae become known, more time will be needed for species identification, and the sensitivity of the biotic index will be increased. It seems unlikely, however, that the total time needed to obtain a biotic index at the species level will increase appreciably since in 1977, when this study was carried out, only 30% of the arthropods could not be identified to species. Time required to make identifications will vary with experience, but anyone who has spent six months or more identifying aquatic insects should be able to identify most genera without consulting a key. Various keys and descriptions were used to make species identifications. When making species determinations it is ad-

TABLE 2. Classification of streams by average of 1977 and 1978 biotic index values with generic biotic index values in parenthesis.

1.75 - Excellent water quality		
0.85 (1.41) Mecan R. #1	1.31 (1.34) Peshtigo R.	1.55 (1.55) Armstrong Cr.
0.86 (1.41) Pine Cr.	1.31 (1.58) Spring Cr.	1.58 (1.59) Namekagon R.
0.87 (1.60) Whittlesey Cr.	1.35 (1.60) Big Roche a Cri	1.61 (1.64) McKenzie Cr.
1.11 (1.52) E. Cranberry R.	1.46 (1.53) Lawrence Cr.	1.73 (1.72) Lit. Jump R.
1.25 (1.58) Sidney Cr.	1.50 (1.78) White R.	1.74 (1.74) Lit. Somo R.
1.30 (1.36) Otter Cr.	1.52 (1.60) N. Br. Levitt Cr.	1.74 (1.87) Rock Cr.
1.76-2.25 - Very good water quality		
1.78 (1.91) Chemical Cr.	1.99 (1.93) Arkansaw R.	2.10 (2.19) St. Croix R.
1.81 (2.03) Mullet R.	2.01 (1.99) Lit. Black R.	2.13 (2.09) Jericho Cr.
1.88 (1.61) Eau Galle R. #1	2.02 (2.07) Milancthon Cr.	2.14 (2.06) Newood R.
1.96 (1.96) Copper Cr.	2.08 (2.03) Mecan R. #2	2.21 (2.20) Onion R.
2.26-2.75 - Good water quality		
2.26 (2.18) Sugar Cr.	2.43 (2.38) Trade R.	2.59 (2.33) Kickapoo R.
2.27 (2.20) Pine R. #2	2.43 (2.28) Neenah Cr.	2.60 (2.64) Wisconsin R. #1
2.38 (1.98) Poplar R.	2.45 (2.33) Sugar R.	2.64 (2.59) Yellow R.
2.41 (2.33) Clam R.	2.46 (2.43) Bluff Cr.	2.74 (2.67) Pine R. #1
2.42 (2.42) Wisconsin R. #4	2.52 (2.45) Missouri Cr.	
2.76-3.50 - Fair water quality		
2.87 (2.70) Narrows Cr.	2.88 (2.81) Wood R.	3.21 (3.06) Wisconsin R. #3
2.88 (2.52) Sheboygan R.	2.97 (2.10) Eau Galle R. #2	3.32 (2.78) Steel Brook
3.51-4.25 - Poor water quality		
4.04 (3.88) Wisconsin R. #2		
4.26 - Very poor water quality		
4.51 (4.48) Beaver Dam R.	4.60 (4.21) Badfish Cr.	

vantageous to work with one genus at a time, identifying species from all streams being studied before making identifications in another genus. Collection of samples, the initial sorting and labeling of specimens, mounting of Chironomidae on slides, and calculation of index values can be done by persons without specialized training, allowing trained personnel to concentrate on identifications. In this study the untrained student was able to sort and label a sample in an average of 33.6 minutes. I was then able to correct sorting errors and make generic identifications in 11.3 minutes, compared to 32.4 minutes when I sorted and labeled in addition to making identifications.

Based on the average of 1977 and 1978 biotic index values, streams were arbitrarily placed into 6 water quality categories (Table 2). In the "excellent" category generic biotic index values averaged 0.21 higher than species values, with the greatest disparities being in streams with the lowest biotic indexes. In the "very good" category, generic biotic index values averaged less than 0.01 higher than species values, and in the "good" category they averaged 0.10 lower. Some of the greatest disparities occurred in the last three categories, where generic biotic index values averaged 0.30 lower than species values. These disparities were due mostly to numerous *Symphitopsyche bifida* group (tolerance value 3) and/or *Simulium vittatum* (tolerance value 4) in these streams and the use of generic tolerance values of 2 and 3 respectively. In the 104 samples collected, generic biotic index values differed from species values by 0.50 or more in 11% of the samples and by 0.25 or more in 31% of the samples. Using only generic identifications could result in the erroneous assessment of the water quality of a stream. When one considers the considerable time required to drive to and from collection sites, in addition to time itemized in Table 1, time needed to make necessary species identifications is small by comparison. I agree with Resh and Unzicker (1975) that species identifications should be made whenever possible. Generic identifications are adequate for calculating a biotic index only when all species in a genus have the same index value or when the objective of the study is to detect severe pollution.

LABORATORY PICKING VS. FIELD PICKING OF SAMPLES

To evaluate streams with the biotic index, it was originally recommended that 100 insects, amphipods, and isopods be picked from the sample in the field while they are still living. The main advantages of this procedure are that living arthropods are easier to see becuase of their movement, and if an inadequate sample is collected, an additional sample can be obtained without having to return to a stream that may be several miles away. The principal disadvantage of live picking is the introduction of a sampling bias, the assumption being that larger and slower moving arthropods will make up a disproportionate share of the sample. This problem can be alleviated if the original sample contains only slightly more than the 100 arthropods that are desired for a sample. However, in a recent study of more than 1,000 Wisconsin streams as well as in other studies, samples were preserved along with debris in the field and the 100 arthropods were picked from the sample in the laboratory. This was done to avoid bias, with the sample placed on a grid and arthropods removed according to a prescribed procedure until the desired sample size was obtained.

A study was carried out to determine how the two methods of picking affect biotic index values.

Materials and Methods

Six samples of 100 arthropods were collected from each of 5 Wisconsin streams in late June 1981 to determine if bias is present in the two sampling procedures and to estimate the efficiency of each procedure. The samples were alternately picked in the field or preserved in alcohol and returned to the laboratory for picking. Because of the scarcity of arthropods, 12 samples of 50 arthropods were collected from Armstrong Creek. The time required to remove arthropods from a sample in the laboratory, sort them into labeled vials, and identify them to genus was recorded so that it could be compared with the time needed to sort and identify field-picked samples in a previous experiment (Table 1). Biotic index values were calculated for all samples and compared with a *t* test. Numbers of individuals collected in each of the 17 most prevalent groups of arthropods were tabulated and compared to determine if a sorting bias existed.

Results and Discussion

Results show that a sampling bias does exist in several families of aquatic insects, and for this reason biotic index values calculated for a given stream can vary depending on the sorting method used. The 10 families in which bias was apparent are ranked in Table 3. Elmidae larvae, especially those of *Optioservus*, were much more abundant in laboratory-picked samples than in field-picked samples. These larvae are small, immobile, and cryptically colored, and thus difficult to find among debris in field-picked samples. Those picked in the field were mostly picked from the net. In the laboratory, after preservation in 70% ethanol and dilution with water in the sorting pan, the larvae became somewhat distended, exposing the pale intersegmental membrane. This made them conspicuous among the debris.

Glossosomatidae larvae were found in only two streams, and were much more abundant in laboratory-picked samples. Living larvae tend to remain in their cryptically colored sand cases and not move, which made them difficult to find in field-picked samples. When placed in ethanol, they vacated their cases and their abdomen turned white, making them conspicuous in laboratory-picked samples.

Brachycentridae larvae, while also cryptically colored, actively moved about and were readily seen when field-picking material. When preserved in ethanol, they mostly retreated into their cases and were difficult to find among the debris.

Elmidae adults, unlike the larvae, were more abundant in field-picked samples. Many clung to the sample net after the debris had been emptied into the sorting pan, and about half of the adult elmids that were collected were picked from the net. In the field the adult beetles tended to crawl to the edges of the sorting pan and their movement made them easy to see. The cryptically colored adults were difficult to see among the debris in samples preserved for laboratory picking.

Living *Hexatoma, Antocha,* and *Dicranota* are cryptically colored and not very active, which made them difficult to find in field-picked samples. When preserved, they became light-colored and easy to find. The remainder of the biases appear to be much less significant. Perlidae, Hydropsychidae, Heptageniidae, and Athericidae are all active and of a relatively large size, making them easy to see and capture in field-picked samples. Because of their general cryptic coloration they were not so readily seen in laboratory-picked samples. Chironomidae larvae, on the other hand, are usually small, and except for those that are red, are cryptically colored and rather difficult to find among the debris unless they become active and try to swim. After preservation in ethanol, most chironomid larvae became lighter in color and were somewhat easier to find among the debris.

There is no doubt that the picking of live samples in the field does produce bias and it is very likely that picking of preserved samples also produces a bias. The more important question is the effect of these biases on the biotic index. In the present test, only in the Mecan River was there a significant difference in biotic index values due to picking procedures (Table 4), but this difference would not have altered our evaluation of that stream (Table 7). Excessive numbers of *Optioservus* larvae in the laboratory-picked samples (149 vs. 6) and *Brachycentrus* larvae in the field-picked samples (86 vs. 24) accounted for this difference. In Armstrong Creek, Milancthon Creek, and the Poplar River, the biotic index of field-picked samples was always slightly lower. The difference of 0.05 in Armstrong Creek is of no consequence; the biases balance out, but biases apparently do exist. This is especially interesting since in this stream each sample contained only 50 arthropods, and in both field- and laboratory-picked samples these were obtained only after a long and careful search. At the time of picking it was assumed that virtually every arthropod had been removed from each sample, yet there were 48 *Glossosoma* in the laboratory-picked samples and only 8 in those picked in the field. There were also 36 *Optioservus* larvae in laboratory-picked samples and only 4 in the field-picked samples. Sixty Hydropsychidae, 48 *Atherix,* and 13 Ephemerellidae were found in field-picked samples, while numbers in laboratory-picked samples were 31, 34, and 6 respectively. Although exhaustive picking tends to reduce biases, it certainly does not eliminate them. The results from this stream strongly suggest a bias in laboratory picking as well as in field picking.

TABLE 3. The degree of bias in laboratory and field-picked samples.

Family Group or Family	No. Arthropods Lab*	Field	Difference	Bias** Ratio	Bias Rank
Perlidae	96	142	+46	+1.47	6
Baetidae	173	176	+3	+1.01	
Ephemerellidae	65	65	0	1.00	
Heptageniidae	40	57	+17	+1.43	8
Other Ephemeroptera	38	38	0	1.00	
Odonata	12	14	+2	+1.17	
Brachycentridae	31	94	+63	+3.03	3
Glossosomatidae	54	12	−42	−4.50	2
Hydropsychidae	245	358	+113	+1.46	7
Corydalidae	38	35	−3	−1.09	
Elmidae adults	38	84	+46	+2.21	4
Elmidae larvae	264	36	−228	−7.33	1
Athericidae	37	48	+11	+1.30	9
Chironomidae	93	73	−20	−1.27	10
Simuliidae	46	54	+8	+1.17	
Tipulidae	51	28	−23	−1.82	5
Gammaridae	54	61	+7	+1.13	
Asellidae	200	202	+2	+1.01	

* Adjusted so that laboratory totals equal field totals.

** Bias ratio is ratio of largest number to smallest.

TABLE 4. Comparison of mean biotic index values for field-picked samples with those of laboratory-picked samples in five streams.

Stream and county	Biotic Index Value: Field	Biotic Index Value: Laboratory	Degrees Freedom	t	Standard Deviation
Armstrong Creek, Forest	1.44	1.49	10	0.85	0.11
Badfish Creek, Rock	4.46	4.25	4	0.63	0.40
Mecan River, Waushara	1.03	1.59	4	6.51**	0.11
Milancthon Creek, Richland	1.90	2.02	4	1.69	0.08
Poplar River, Clark	2.56	2.70	4	1.40	0.12

** $P = < 0.01$

In the Poplar River there were significantly greater numbers of Perlidae, Heptageniidae, Hydropsychidae, and Chironomidae in field-picked samples while Baetidae and *Asellus* were significantly more prevalent in laboratory-picked samples. This resulted in an insignificantly lower value for field-picked samples. The lower average value for field-picked samples in Milancthon Creek was due to one sample that contained 41 *Baetis vagans* (tolerance value = 1). In Badfish Creek field-picked samples averaged slightly higher than laboratory-picked samples due almost entirely to one laboratory-picked sample that contained only 54 arthropods, and only 17 *Asellus* (tolerance value = 5). The Badfish Creek samples had large amounts of filamentous algae, and it was difficult to find and remove arthropods from this algae. In the field, *Asellus* and other arthropods tended to crawl out of the algae where they could be readily collected.

In the previous study, the time required to collect a sample, pick 100 arthropods in the field, and identify them to genus required an average of 54 minutes. In the four streams in this study where samples of 100 arthropods were collected, it took an average of 51 minutes to pick the arthropods in the laboratory and identify them to genus. It took an average of about 5 minutes to collect these samples, so there appears to be no significant time advantage for either method.

The results of this study indicate that although differences in biotic index values may occur as a result of the method used to pick the sample, these differences are usually not significant and do not affect the evaluation of the stream. The reason for laboratory picking of samples was to reduce bias, but biases apparently result from laboratory picking as well as from field picking of samples. The advantages of laboratory picking are a possible reduction of biases and the use of laboratory time instead of valuable field time for picking. Advantages of field-picking include better condition of specimens, especially mayflies, a break in the tedium of picking samples (interspersed with sampling and driving), and the ability to return for an additional sample if the first is inadequate. Because of the fear of an inadequate sample being returned to the laboratory for picking, additional sampling time is often spent to assure that the sample is adequate. This results in more time being spent in the collecting and processing of laboratory-picked samples than field-picked samples. More Chironomidae are collected in samples picked in the laboratory, and because they have to be slide-mounted for identification, this adds to the time and expense of processing samples picked in the laboratory. If care is taken that samples contain no more than 200 arthropods and that the arthropods picked from the sample are representative of the sample, picking of the sample in the field appears to be the desirable procedure in terms of time spent and results achieved.

ARTIFICIAL SUBSTRATE SAMPLERS AS AN ALTERNATIVE SAMPLING PROCEDURE

The biotic index relies upon samples from the riffle community, but some streams do not have riffles, or runs of rock or gravel. In these streams sampling of snags of debris in the current has been the alternative method for collecting arthropods with a net. Deep streams are almost impossible to sample. Artificial substrate samplers have been employed in biological monitoring (Weber 1973), and offer an alternative sampling method for deep streams and streams without riffles or snags. Rock basket samplers have been most widely used, and in this study a modification of the rock-basket sampler (Hilsenhoff 1969) was compared with net samples.

Materials and Methods

Three samplers were placed in each of 6 stream sites between 3 and 10 June 1980. All streams had been studied previously (Hilsenhoff 1972, 1977), and ranged from severely polluted to unpolluted. The Mecan River was the only stream without a rock or gravel riffle; it had a shifting sand bottom. One stream, the Newood River, was sampled at 2 sites about 2 miles apart, the downstream site having a greater gradient and faster current. On 4 to 6 August, and again from 29 October to 6 November, 3 net samples were collected at each site along with a sample from each sampler. The net samples were collected from a riffle according to standard procedures described earlier, with a maximum of 15 minutes being spent in the removal of arthropods from the net and pan. In the Mecan River, where no riffle was present, samples were collected from snags of debris. Sampler samples were collected as described by Hilsenhoff (1969) to minimize loss of arthropods due to disturbance, and they were picked in the field until 100 arthropods were collected or until 15 minutes had elapsed. Biotic index values were calculated for all samples and compared by analysis of variance.

Results and Discussion

In October there was no significant difference between net and sampler samples, but in August net samples in half of the streams produced significantly higher biotic index values than sampler samples (Table 5). In the Wisconsin River and Newood River this was due to disproportionately large numbers of hydropsychid caddisflies with a tolerance value of 3, *Cheumatopsyche* in the Wisconsin River and *Symphitopsyche bifida* group in the Newood River. In all streams there was only minimal colonization of samplers by Hydropsychidae during the early summer exposure period, but significant colonization in late summer and autumn. This was most likely due to oviposition periods, because large hydropsychid larvae do not drift and would be unlikely to colonize samplers. In the Pine River, the samplers were always poorly colonized, and disproportionately large numbers of *Acroneuria lycorias* (tolerance value =0) in the samplers in August led to a significantly lower biotic index value. In the Mecan River, where net samples were collected from snags of debris instead of riffles, biotic index values of net and sampler samples were very similar in both months. This suggests that sampler samples most closely approximate net samples from debris, which is logical since samplers are placed in the current and accumulate debris.

TABLE 5. Comparison of biotic index values and average sample sizes of sample's taken by net and artificial substrate samplers in 6 Wisconsin streams.

	August		October	
	Net	Sampler	Net	Sampler
Badfish Creek, Rock Co.				
Avg. biotic index	3.99*	4.33[1]	3.53	3.78
Avg. sample size	80	74	107	107
Wisconsin River, Lincoln Co.				
Avg. biotic index	2.71*,**	2.15	2.21	2.39
Avg. sample size	104	54	99	103
Pine River, Forest Co.				
Avg. biotic index	1.89*,**	1.43	1.08	1.13
Avg. sample size	108	24	102	34
Newood River, Lincoln Co.				
Avg. biotic index	2.33*,**	1.61	1.50	1.82
Avg. sample size	88	65	101	71
Newood River - Hwy E				
Avg. biotic index	1.72	1.63	1.15	1.39
Avg. sample size	88	75	101	67
Mecan River, Marquette Co.				
Avg. biotic index	1.87	1.86	1.52	1.63
Avg. sample size	90	53	86	100
Pooled data from all streams				
Avg. sample size	93	58	99	80
S.D. biotic index[2]	0.110	0.269	0.098	0.229

* Significantly different from October net samples.
** Significantly different from August sampler samples.
[1] Significantly different from October sampler samples.
[2] Standard deviation of pooled samples from their means.

In all of the streams, biotic index values of net samples, and usually also of sampler samples, were higher in August than in October. In most of the streams these differences were significant. This points to the urgent need to develop a reliable seasonal correction factor for the biotic index.

The pooled standard deviation (s) of sampler samples was more than twice as great as that of net samples in both of the months. This could be partly due to the smaller average sample size of sampler samples. It is more likely due to a tendency of aquatic insects to have a clumped distribution. The net samples were taken by disturbing an area of the bottom that was several times greater than that of a sampler and contained a variety of substrates as compared to the homogeneous substrate of the sampler.

In streams with shifting sand bottoms and no snags of debris, or in streams too deep to have riffles or runs that can be sampled with a net, artificial substrate samplers present a viable alternative sampling method. The samplers used in this study would not be satisfactory because they rest on the bottom and become buried in streams with shifting sand bottoms and cannot be retrieved from deep streams. A "Bar-B-Q basket" sampler (Mason et al. 1967) suspended from bridges would be large enough to obtain an adequate sample because it contains about 20 lb of rocks compared to the 8 lb in the samplers used in this study, but this sampler would not solve the problem of clumped distribution. A pooled sample from 4 or 5 small rock-in-basket samplers suspended from a bridge is a better alternative if the samplers can be concealed from the curious public for the 8-week period needed for colonization, and if they can be enclosed during retrieval to prevent the escape of arthropods.

RELIABILITY OF SAMPLES

Previously I concluded that a sample of 100 arthropods was adequate for assessing water quality with the biotic index (Hilsenhoff 1977). In 1976 Kaesler and Herricks also concluded that a sample of 100 insects was sufficient when using diversity indexes to evaluate the water quality of streams, and that larger sample sizes were not warranted. To reaffirm the reliability of a sample size of 100, and to determine if sampling at an alternate site on the same stream would affect the biotic index, 9 streams were sampled late in the spring of 1978.

Materials and Methods

From 30 May to 13 June 1978, 6 samples of 100 arthropods were collected from riffle areas in each of 9 streams. One set of 3 samples was collected at the same site sampled previously (Hilsenhoff 1977), and the other set at a riffle some distance away. An analysis of variance was used to determine if there was a significant difference between sites within the same stream. In addition, a standard deviation was calculated from 105 pooled sums of squares obtained from this test and all field-picked riffle samples in previously reported tests.

Results and Discussion

In two streams there was a highly significant difference between samples (Table 6). In the remaining streams there were no significant differences. A difference in the samples from the Newood River, a wilderness stream in Lincoln County, was anticipated. At the upstream site there are infrequent riffles interspersed between runs with a slow current. The water originates in a large swamp and significant amounts of organic matter are sometimes washed into the stream, apparently causing periodic depressed dissolved oxygen levels. The steeper gradient below the upstream site provides sufficient

TABLE 6. Comparison of biotic index values for two sites on the same stream.

Stream and County	Biotic Index	Location of Alternate Site	Mean Biotic Index
Badfish Creek, Rock	4.37	100 yd upstream	4.30
Eau Galle River, Dunn	2.91	100 yd downstream	3.29**
Mecan River, Waushara	0.94	100 yd upstream	0.86
Milancthon Creek, Richland	2.08	100 yd upstream	2.13
Newood River, Lincoln	2.15	2 miles downstream	1.63**
Onion River, Sheboygan	2.34	50 yd upstream	2.22
Otter Creek, Sauk	1.48	1/4 mile upstream	1.47
Sugar River, Dane	2.38	100 yd upstream	2.27
Trade River, Burnett	2.28	50 yd downstream	2.33

** $P < 0.01$

$LSD_{.95} = 0.19$ $LSD_{.99} = 0.26$ S.D. = 0.115

riffles and oxygen to reaerate the stream at the sampling site 2 miles downstream, accounting for a much lower biotic index at that site. The phenomenon of occasionally elevated biotic index values resulting from depressed oxygen levels in wilderness streams that originate in swamps was noted previously by Joe Eilers (pers. comm.). It most frequently occurs after periods of heavy rain and flooding.

A highly significant difference in biotic index was also encountered in the Eau Galle River in Dunn County, and this was not expected. This sampling site on the Eau Galle River is about 100 yd below a hydroelectric dam, where there is significant aeration of the water as it passes through turbines or over a high spillway. Effects of decomposition of organic matter produced in the impoundment would be more prominent farther downstream, and would account for a significantly higher biotic index at the downstream site.

The standard deviation from pooled sums of squares in this and all other replicated experiments in which 100 arthropods were collected with a net and picked in the field, was 0.098. This means that 95% of biotic indexes calculated from a sample of 100 arthropods should be within 0.19 of the true index value, and 99% should be within 0.25.

A previous test in which field-picked samples were compared with laboratory-picked samples provided the only set of samples with replicates of 50 arthropods instead of 100. There were 6 replicates of 50 in the samples from Armstrong Creek, and the data from these replicates were combined in all possible combinations to produce sample sizes of 100, 150, and 200. Biotic index values were calculated for all sample sizes. In the field-picked samples the standard deviation was compared for all sample sizes and it was 0.071 for replicates of 50, and 0.035, 0.031 and 0.026 for replicates of 100, 150, and 200 respectively. This indicates that a sample size of 50 arthropods is only half as reliable as the standard sample of 100, and reaffirms that the additional time needed to collect and process larger samples is probably not justified because sample sizes of 150 or 200 did not significantly increase the reliability of the sample.

PROBLEMS

The biotic index has been shown to be a rapid, sensitive, and reliable method for evaluating the water quality of streams, but there are problems involved with its use and they must be considered when interpreting results. Solutions to some of the problems are forthcoming, while others may not be realized for several years. Three of the problems I consider major, and will discuss them first.

Need for Keys to Species. In several genera of aquatic insects, especially in the Ephemeroptera, Trichoptera, and Diptera, it is not yet possible to identify larval stages beyond genus. In genera where all species have the same tolerance value, this is of no consequence, but in genera where tolerance values of the species differ, it is important to be able to recognize each species. In the mayfly genus *Caenis* and the caddisfly genera *Cheumatopsyche* and *Symphitopsyche* we have several species that cannot be separated, and it is obvious from our experience in collecting these genera that tolerance values of the various species range from 1 to 4 within each genus. Presently all unidentifiable species in these genera have been assigned a value of 3, which tends to raise calculated biotic index values of clean streams and lower calculated biotic index values of polluted streams. Since all of these genera frequently occur as a dominant segment of a stream's fauna, the problem is serious. In the genus *Symphitopsyche*, it is only species in the *bifida* group that cannot be identified, and Patricia Schefter, a graduate student at the University of Toronto, plans to publish keys to species within a year. *Cheumatopsyche* larvae on the other hand seem to present a more serious challenge to taxonomists. Several efforts have been made to develop larval keys, but no one has succeeded and nobody is presently working on this problem. A study of *Caenis* mayfly larvae was initiated recently by Arwin Provonsha at Purdue University, but it may be several years before a species key can be developed. Taxonomic problems exist in many other genera, but only on the rare occasions when these genera are a dominant segment of the fauna may these problems significantly affect calculated biotic index values.

Correction Factors for Current and Temperature. It has been shown in laboratory studies (Lloyd Lueschow, DNR, pers. comm.) that increased current and lowered water temperature enhance an arthropod's ability to withstand decreased dissolved oxygen levels. At lower water temperatures the metabolism of arthropods is slowed and their need for oxygen is decreased, thus they can tolerate lower levels of dissolved oxygen. Similarly, as current is increased, more water passes over the respiratory organs of arthropods, exposing them to more dissolved oxygen, and this enables them to survive at lower levels of dissolved oxygen. Correction factors for maximum water temperature and maximum current at the sampling site need to be developed to better relate biotic index values to minimum oxygen levels and water quality. The critical time for both parameters is usually midsummer.

Seasonal correction factors. After sampling 53 streams 4 times during a year, a seasonal correction factor was suggested (Hilsenhoff 1977), but it needs refinement. In the study in which the use of samplers was tested (Table 5), biotic index values obtained by net samples were always higher for August than October, and in two-thirds of the streams the differences were statistically significant. The average difference of 0.59 is of such a magnitude that it would seriously jeopardize interpretation of results if seasonal differences in biotic index values were not taken into consideration. Seasonal variations in the biotic index are probably mostly a function of water temperature, which affects emergence, egg hatching, diapause, and other parts of the life cycle of aquatic insects. In summer, when dissolved oxygen levels tend to be lowest, resistant species and resistant life stages tend to predominate. Life cycles are related to seasonal temperature patterns, which do not always proceed on the same schedule every year, and thus seasonal correction factors must be tied to phenological events rather than to the calendar. Since streams have wide daily temperature fluctuations, the water temperature of large monomictic lakes appears to be the best phenological event upon which to base a seasonal correction factor.

Assignment of tolerance values. Tolerance values were initially assigned to each species empirically, and adjustments were made when studies of groups of streams suggested they were necessary. An insect species with an assigned tolerance value of 0 that is found frequently in streams in which all other species have a value above 2 obviously has an erroneous value that must be changed. Many such changes were made after a study of data from 563 streams that were sampled in the spring and autumn of 1979. An additional 455 streams were sampled in the spring and autumn of 1980, and the data from all 1,018 streams should be computerized to facilitate the adjustment of tolerance values assigned to each species. This may also make it possible to refine the index by expanding the present 0-5 scale to 0-10.

Other considerations. Several other factors may affect the biotic index, and although these effects presently appear to be minor, future research may prove otherwise. Adjustments or correction factors may be needed when evaluating laboratory-picked samples, samples collected with artificial substrates, or samples collected from snags of debris instead of from a riffle. Corrections may also be needed for various substrates that make up the riffle, stream size, shaded vs. open streams, stream depth, and perhaps other factors not yet considered.

RECOMMENDED SAMPLING PROCEDURE

1. With a D-frame aquatic net, sample a site where flow is most rapid and the substrate is composed of gravel or small stones. This is best accomplished by placing the net against the substrate and disturbing the substrate immediately upstream from the net.
2. Sample until you have collected somewhat in excess of 100 arthropods, but be careful not to collect more than 200 because large numbers will tend to bias the sample when sorting.
3. Place the contents of the net in a shallow pan with a small amount of water.
4. Remove arthropods clinging to the net. Do not bias the sample by collecting more than 20 arthropods from the net.
5. Remove arthropods from the pan with a curved forceps until you have collected 100, including those removed from the net. Strive for variety; do not pick certain types of arthropods to the exclusion of others. Do not collect Hemiptera or Coleoptera, except Gyrinidae larvae and Dryopoidea. Do not collect individuals less than 3 mm long, except Hydroptilidae larvae and Elmidae adults.
6. Preserve all arthropods in 70% alcohol for identification to genus or species in the laboratory.
7. If an area of gravel or small stones cannot be found for collection of the sample, sample debris in the fastest current. Leaves, grasses and other debris clinging to branches or snags are very good sources of arthropods.
8. If the original sample does not contain 100 arthropods, collect a second sample, but do not spend more than 30 minutes collecting and picking samples. A complete absence of arthropods in a stream that contains good habitat is an indication of severe pollution.
9. Streams with no perceptible current cannot be evaluated with the biotic index at this time. Streams that cannot be sampled because of their depth or lack of suitable substrate can be sampled with artificial substrate samplers. Suspend rock-in-basket samplers from bridges or overhanging tree branches, and leave them in the stream at least 8 weeks. They should be hidden from the curious public, and before removing them they must be enclosed to prevent the escape of arthropods.

Alternative Procedure for Steps 3-5

Alternative Step 3 - Place the contents of the net in a pint jar and cover with 80% alcohol as a preservative. Include all arthropods clinging to the net.
Alternative Step 4 - In the laboratory place the contents of the jar in a large pan marked with a grid, and spread the contents evenly over the bottom of the pan.
Alternative Step 5 - Systematically remove arthropods from the grid, section by section, removing all arthropods from a section before removing any from the next. Remove and preserve 100 arthropods. Do not collect Hemiptera or Coleoptera, except Gyrinidae larvae and Dryopoidea. Do not collect individuals less than 3 mm long, except Hydroptilidae larvae and Elmidae adults.

IDENTIFICATION

Insect genera can be identified by using the keys in *Aquatic Insects of Wisconsin* (Hilsenhoff 1981), and references to the most recent species keys will also be found in that publication. However, since many of the species keys are not readily available, those that are needed for biotic index calculations have been modified for Wisconsin and are reproduced here. Amphipods may be identified by using Holsinger (1972), and isopods by using Williams (1972).

KEY TO NYMPHS OF *PERLINELLA*

1 Anterior ocellus absent or indicated by a slight depression; anal gills small; entire insect a uniform light brown ..... *ephyre*
Anterior ocellus present, although inconspicuous in small nymphs; anal gills long; head and thorax indistinctly patterned ..... *drymo*

KEY TO NYMPHS OF *ISOPERLA*

1 Second tooth of lacinia absent (Fig. 1-A) ..... *nana*
Second tooth of lacinia present 2

2 (1) Truncate distal end of lacinia covered with a dense brush of setae (Fig. 1-B); abdominal markings, if present, longitudinal and never transverse..... *lata*
Lacinia variable, but without a dense brush of setae distally 3

3 (2) Lacinia with a tuft of setae below second tooth (Figs. 1-C,D) 4
Lacinia with setae scattered below second tooth, none clustered in a tuft (Figs. 1-E,F) 6

4 (3) First tooth of lacinia about as long as outer edge of ovate basal portion of lacinia (Fig. 1-C); no paired dark spots on abdominal or thoracic terga ..... *cotta*
First tooth of lacinia much shorter than outer edge of elongate basal portion (Fig. 1-D); paired dark spots on either abdominal or thoracic terga 5

5 (4) Eight dark spots on each abdominal tergum; thoracic terga mottled with light and dark areas; dark bar on anterior portion of frontoclypeus enclosing a light area just anterior to median ocellus ..... *richardsoni*
Dark spots absent from abdominal terga; each thoracic tergum pale with paired dark spots; no dark bar on anterior portion of frontoclypeus ..... *frisoni*

6 (3) Abdominal terga transversely banded or pale anteriorly and dark posteriorly, especially on posterior terga (telescoping of segments may give false appearance of banding); rarely dark nymphs are evenly colored, but dark pigment extends ventrally well down onto posterior margin of 9th sternum 7
Abdomen with longitudinal stripes, light spots, or evenly colored; if evenly colored, dark pigment does not extend onto 9th sternum 8

7 (6) Distal end of lacinia truncate with several strong setae (Fig. 1-E) ..... *marlynia*
Distal end of lacinia not at all truncate, with only a few strong setae on margin (Fig. 1-F) ..... *signata*

8 (6) Large, quadrate, nearly square light area anterior to median ocellus; dark bands on femur and tibia near their articulation..... *slossonae*
Light area anterior to median ocellus, if present, rounded or W-shaped; no dark bands on femur and tibia near their articulation 9

9 (8) Distinct W-shaped pale area anterior to median ocellus, extending almost to antennae, and often posteriorly to lateral ocelli and compound eyes; abdominal terga each with eight white spots or solidly colored ..... *clio*
Pale area near median ocellus rounded, indistinct, or absent, but never distinctly W-shaped; abdominal terga with longitudinal stripes, except on very immature nymphs 10

10 (9) Pale mark immediately anterior to median ocellus indistinct or lacking; numerous conspicuous freckle-like spots on abdomen, especially on posterior sterna; dark longitudinal abdominal stripes with very narrow pale borders ..... *dicala*
Distinct pale mark immediately anterior to median ocellus; conspicuous freckle-like spots absent; longitudinal stripes, if present, with wide pale borders 11

11 (10) Wingpads with dark, conspicuous setae; veins in wingpads colored similarly to background; dark spots on abdominal terga lacking or inconspicuous..... *transmarina*
Wingpads with pale inconspicuous setae; pale veins visible in dark-colored areas of wingpads; 8 dark spots on each abdominal tergum .... *bilineata*

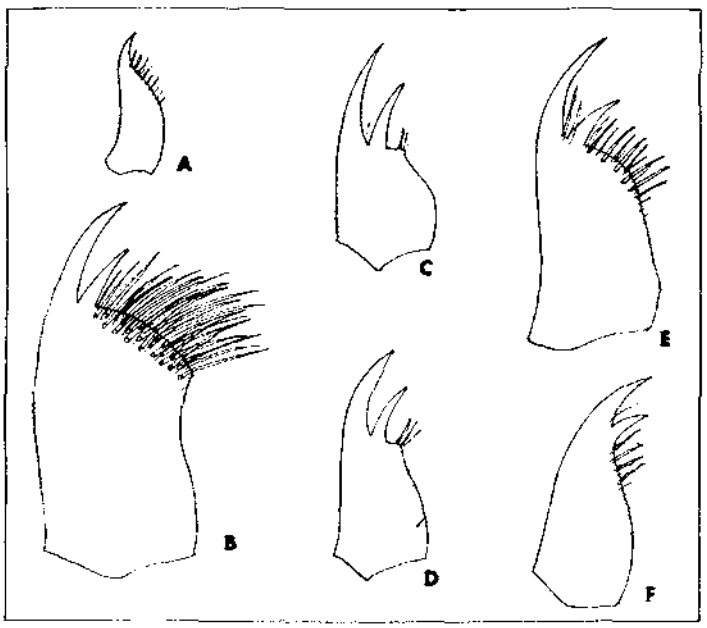

FIGURE 1. *Isoperla*. Lacinia of: A - *I. nana*; B - *I. lata*; C - *I. cotta*; D - *I. richardsoni*; E - *I. marlynia*; F - *I. signata*.

KEY TO NYMPHS OF *BAETIS*

1 Nymph with only two caudal filaments..... *amplus*
Nymph with three caudal filaments, the middle one often shorter..... 2

2 (1) Caudal filaments uniformly colored, without bands.. 3
Caudal filaments with light or dark bands at middle or apex 4

3 (2) Abdominal terga brown, often with a pale median stripe; abdominal terga 10 and sometimes 5 may be pale ..... *brunneicolor*
Abdominal terga without a pale median stripe; terga 5, 9 and sometimes 10 are usually paler than other terga ..... *vagans*

4 (2) Caudal filaments with dark crossbands at or near middle 5
Caudal filaments without dark crossbands at or near middle 11

5 (4) Tibia with a wide dark band at middle; gills on abdominal segment 7 lanceolate (Figs. 2-A, B) 6
Tibia unbanded or banded only at apex; gills on abdominal segment 7 rounded 7

6 (5) Gills on segment 7 sharply pointed at apex, very narrow (Fig. 2-A) ..... *pygmaeus*
Gills on segment 7 elongate, but not sharply pointed (Fig. 2-B) ..... *macdunnoughi*

7 (5) Abdominal terga uniformly dark, each with an interior and posterior median white dash forming an interrupted or continuous pale median line on abdomen (Fig. 2-C) ..... *frondalis*
Abdomen usually with some pale terga, if uniformly dark, without a pale interrupted median line on abdomen 8

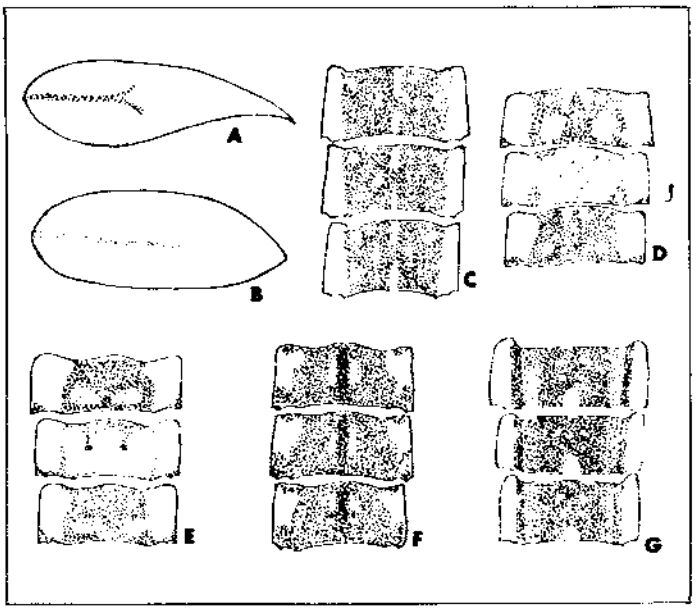

FIGURE 2. *Baetis*. Seventh abdominal gill of: A - *B. pygmaeus;* B - *B. macdunnoughi.* Abdominal terga 4, 5, and 6 of: C - *B. frondalis;* D - *B. intercalaris;* E - *B. flavistriga;* F - *B. propinquus;* G - *B. longipalpus.*

8 (7) Cerci banded at or near apex; a dark band at articulation of tarsi and tibiae 9
Cerci not banded near apex; tarsi and tibiae without dark marks 10

9 (8) Abdominal tergum 10 and posterior of 5 often pale; abdominal terga with distinct mid-anterior paired, pale, oblique dashes and dots, often obscure in terga 1, 9, and 10 and in darkly pigmented specimens (Fig. 2-D); tarsi not banded at apex ..... *intercalaris*
Abdominal tergum 9 usually pale; mid-anterior paired, pale, oblique dashes and dots indistinct or absent, when present a faint longitudinal line often between paired dashes and dots (Fig. 2-E); a dark band at apex of tarsi ..... *flavistriga*

10 (8) Abdominal terga uniformly dark, 10 sometimes pale; large, paired, pale dashes and dots in basal half of each tergum and usually a darkened area in between (Fig. 2-F); gills tracheated with some branching ..... *propinquus*
Abdominal tergum 7 often pale like segment 10; only very tiny pale dashes and dots in basal half of each abdominal tergum, with a median pale spot at posterior margin (Fig. 2-G); gills without trachea or with only a hint of tracheation ..... *longipalpus*

11 (4) Caudal filaments tan, with a dark brown apical band on cerci; gills absent from abdominal segment 1 ..... *hageni*
Caudal filaments brown, with a white apical band; gills on abdominal segments 1-7 ..... Species C

KEY TO NYMPHS OF *CLOEON*

1 All gills single, without a recurved dorsal flap ..... *alamance*
At least basal pairs of gills with a recurved dorsal flap ..... (all other species)

KEY TO NYMPHS OF *PSEUDOCLOEON*

1 Cerci alternately banded light and dark; terga tan with central white dash and usually also submedian white dots on anterior ..... *parvulum*
Cerci unbanded or banded at middle; terga marked otherwise 2

2 (1) Cerci unbanded 3
Cerci banded at middle and usually also at tip 4

3 (2) Short, chunky species with broad thorax; abdominal terga tan, lighter posteriorly, especially on segments 9 and 10 ..... *carolina*
Elongate species; abdominal terga 4 and 8-10 paler than other terga ..... *cingulatum*

4 (2) Abdominal tergum 5 and sometimes 9 much darker than other terga; other terga pale, with 1 and 6-9 sometimes darker ..... Species A
Abdominal tergum 5 not darker than other terga ..5

5 (4) Abdominal terga similarly colored and usually with a median longitudinal pale stripe; terga usually with 2 pairs of submedian dark spots; abdominal sterna often with a black median spot; gills well tracheated ..... *punctiventris*
Abdominal terga without a pale median stripe; black spots never present in middle of abdominal sterna, but basal brown or purple spots may be present 6

6 (5) Male with abdominal terga 3, 4 and 8-10 pale; female uniformly tan with a pair of submedial white spots and a pale central spot on each middle abdominal tergum ..... *dubium*
Abdominal terga 1-2 and 6-7 dark with a pair of posterior submedian white spots; other terga pale with two pairs of submedian dark spots ... *myrsum*

KEY TO NYMPHS OF *EPHEMERELLA*

1 Middle abdominal terga each with a pair of prominent upward projecting spines (Figs. 3-A,B) 2
Middle abdominal terga without such spines, at most a very small pair of posterior projecting spines (Fig. 3-C) 3

2 (1) Spines long, sharp, and found on segments 1-8 (Fig. 3-A); a pale stripe on abdomen between spines ..... *needhami*
Spines moderately long, sharp, and found on segments 2-9 (Fig. 3-B); abdomen without a longitudinal pale stripe ..... *subvaria*

3 (1) Middle abdominal terga with paired tubercles that often result in a small spine or rearward projection on posterior margin of each tergum 4
Middle abdominal terga without paired tubercles; posterior margin of each tergum straight or evenly curved 7

4 (3) Tibiae and tarsi without dark bands; tail filaments without dark bands ..... *catawba*
Tibiae and tarsi with dark bands; tail filaments with or without dark bands 5

5 (4) Middle abdominal terga each with a pair of small tubercles from which a tiny spine projects rearward (Fig. 3-C); caudal filaments with dark bands near middle and at apex ..... *invaria* or *rotunda*
(Spines more prominent in *rotunda*, extremely small in *invaria*)

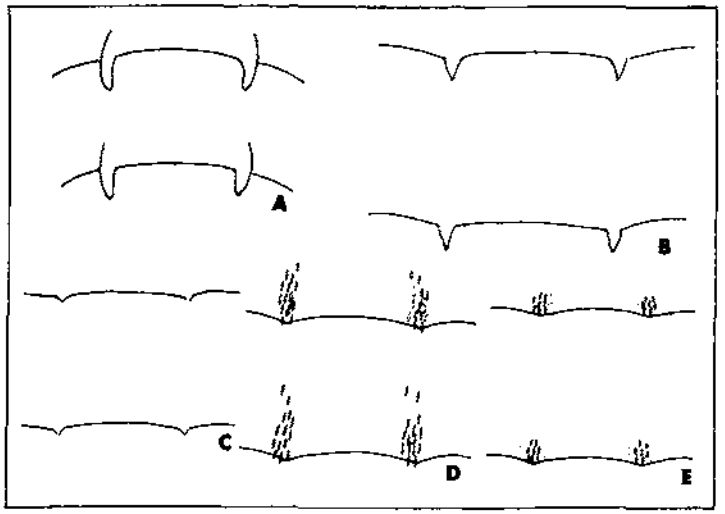

FIGURE 3. *Ephemerella.* Spines and tubercles on abdominal terga 5 and 6 of: A - *E. needhami;* B - *E. subvaria;* C - *E. invaria;* D - *E. aurivillii;* E - *E.* Species A.

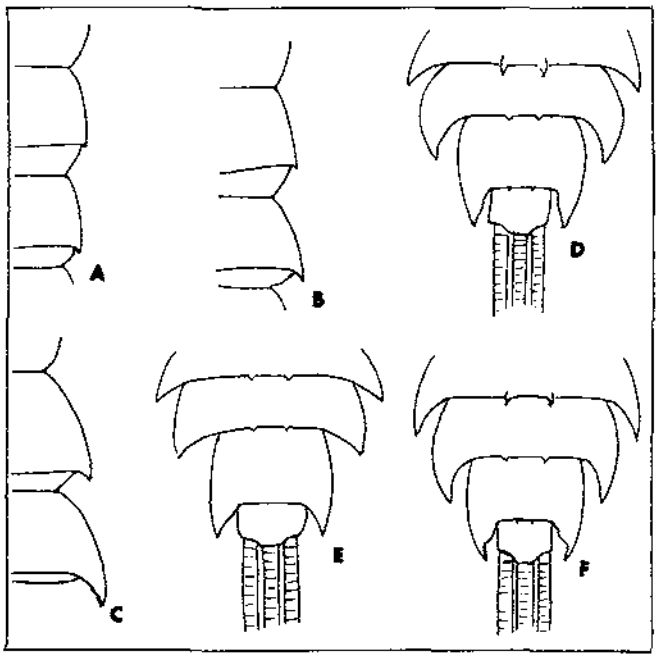

FIGURE 4. *Eurylophella.* Right half of abdominal segments 2 and 3 of: A - *E. bicolor;* B - *E. aestiva;* C - *E. lutulenta.* Abdominal terga 7 to 10 of: D - *E. minimella;* E - *E. bicolor;* F - *E. funeralis.*

Middle abdominal terga each with a pair of distinct tubercles covered with spicules, but without rearward projecting spines (Figs. 3-D,E); bands on caudal filaments variable 6

6 (5) Tubercles very prominent with long spicules (Fig. 3-D); caudal filaments with dark bands near middle and at apex..... *aurivillii*

Tubercles prominent with short spicules (Fig. 3-E); caudal filaments with dark bands mostly in basal half or absent.....Species A

7 (3) Caudal filaments without dark bands, rarely with pale bands near middle..... *excrucians*

Caudal filaments with dark bands..... *dorothea*

KEY TO NYMPHS OF *EURYLOPHELLA*

1 Posterolateral projections barely discernible on abdominal segment 2 and poorly developed on segment 3 (Fig. 4-A); occipital tubercles minute or absent in both sexes 2

Posterolateral projections poorly developed on abdominal segment 2 and very well developed on segment 3 (Fig. 4-B,C) 3

2 (1) Paired tubercles on abdominal terga long and thin; tubercles moderately developed on terga 8-9 (Fig. 4-D) ..... *minimella*

Paired tubercles on abdominal terga short and thick; tubercles poorly developed or absent on *terga* 8-9 (Fig. 4-E) ..... *bicolor*

3 (1) Inner margin of posterolateral projections on segment 9 distinctly incurved (Fig. 4-F); paired tubercles on abdominal terga 8-9 well developed ..... *funeralis*

Inner margin of posterolateral projection on segment 9 not incurved (Fig. 4-E); if slightly incurved, paired tubercles on abdominal terga 8-9 poorly developed.....4

4 (3) Paired tubercles on abdominal terga 1-3 long and blunt, distinctly curved downward apically; occipital tubercles well developed in females, not as well developed in males..... *temporalis*

Paired tubercles on abdominal terga 1-3 short, blunt or sharp; occipital tubercles moderately developed in females, less so in males 5

5 (4) Posterolateral projections poorly developed on abdominal segment 2 and moderately developed on segment 3 (Fig. 4-B); paired tubercles on abdominal terga 8-9 small but distinct; fine back dots absent from body ..... *aestiva*

Posterolateral projections moderately developed on abdominal segment 2 and well developed on segment 3 (Fig. 4-C); paired tubercles minute or absent on abdominal terga 8-9; body and appendages sprinkled with fine black dots..... *lutulenta*

KEY TO NYMPHS OF *SERRATELLA*

1 Abdominal terga without paired, submedian tubercles; maxillary palpi absent ..... *deficiens*

Abdominal terga with paired, submedian tubercles; maxillary palpi present.....2

2 (1) Head, thorax, and legs with long hairs; abdominal terga with tubercles on segments 3 to 8 .. *sordida*

Head, thorax, and legs without long hairs; abdominal terga with tubercles on segments 2 to 8..... *frisoni*

KEY TO NYMPHS OF *HEPTAGENIA*

1 Seventh pair of gills biramous, containing plate and tuft elements; claws not pectinate.....2

Seventh pair of gills uniramous, only plate element present; claws pectinate.....4

2 (1) *Venter of abdomen with dark marking on posterior* edge of ninth sternum only ..... *flavescens*

Dark markings present on lateral margins of ninth sternum and usually present on anterior sterna ...3

3 (2) Abdominal terga 4 and 8 with a pair of pale submedian streaks (Fig. 5-A) ..... *diabasia*

Abdominal terga 4 and 8 with a pale median spot (Fig. 5-B) ..... *pulla*

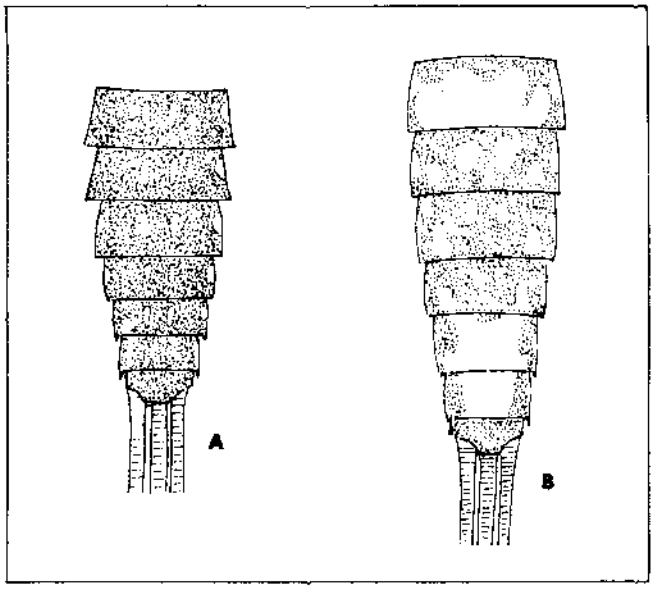

FIGURE 5. *Heptagenia.* Abdominal terga 4 to 10 of: A - *H. diabasia;* B - *H. pulla.*

4 (1) Base of caudal filaments unicolorous, white or nearly so ..... ***lucidipennis***
Base of caudal filaments distinctly darkened at articulations ..... ***hebe***

KEY TO NYMPHS OF *STENONEMA*

1 Posterior abdominal sterna each with a pair of rounded spots, which are most prominent posteriorly (Fig. 6-A); gills on abdominal segments 1-6 rounded ..... ***femoratum***
Abdominal sterna marked otherwise, or unmarked; gills on abdominal segments 1-6 truncate 2

2 (1) Abdominal sterna 3-8 with dark transverse bands (Figs. 6-B,C) 3
Abdominal sterna marked otherwise or unmarked 4

3 (2) Dark bands at posterior edge of abdominal sterna (Fig. 6-B) ..... ***vicarium***
Dark bands at anterior edge of abdominal sterna (Fig. 6-C) ..... ***mediopunctatum***

4 (2) Abdominal sternum 8 with median brown spot; sternum 9 with a U-shaped mark (Fig. 6-D) ..... ***modestum***
Abdominal sterna without markings or with pale markings laterally 5

5 (4) Abdominal terga 7, 8, and 9 with a median pale V-shaped mark (Fig. 6-E) ..... ***integrum***
Pale marks on abdominal terga 7, 8, and 9 not forming a V (Figs. 6-F,G) 6

6 (5) Abdominal terga mostly dark, with various pale markings; terga 8 and 9 often light in center and terga 5-7 often with paired submedian pale spots (Fig. 6-F) ..... ***terminatum***
Abdominal tergum 7 distinctly lighter than terga 6 and 8 (Fig. 6-G) 7

7 (6) Abdominal tergum 9 dark; mature nymphs with a pale transverse band at base of dark wingpads (Fig. 6-H) ..... ***exiguum***
Abdominal tergum 9 paler than terga 8 and 10; nymphs never with a pale transverse band at base of wingpads ..... ***pulchellum***

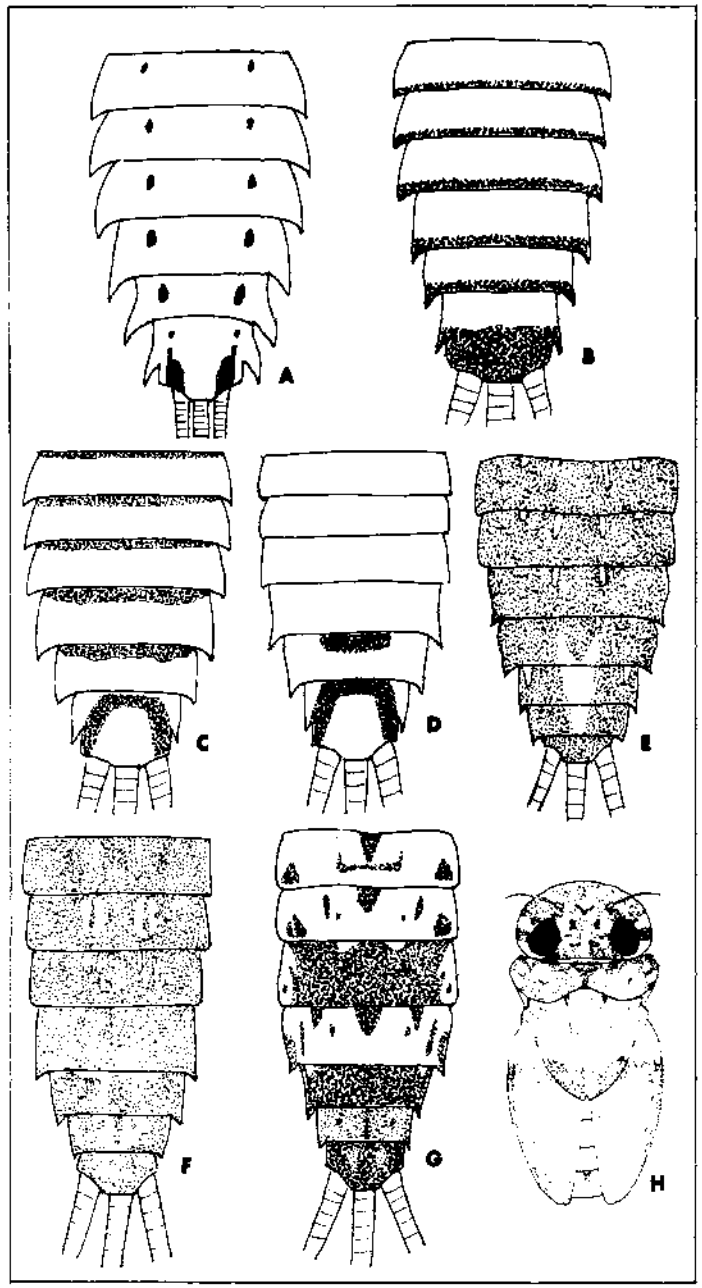

FIGURE 6. *Stenonema.* Abdominal sterna 4 to 9 of: A - *S. femoratum;* B - *S. vicarium;* C - *S. mediopunctatum;* D - *S. modestum.* Abdominal terga 4 to 10 of: E - *S. integrum;* F - *S. terminatum;* G - *S. pulchellum.* H - Head and thoracic terga of *S. exiguum.*

KEY TO NYMPHS OF *ARGIA*

1 Caudal lamellae with a marginal fringe of stiff bristles mixed with fine long hairs toward apex ..... ***violacea***
Caudal lamellae without stiff marginal setae or with only a few near base 2

2 (1) Caudal lamellae broadly rounded distally, with a minute filament at tip, uniformly dark except along the apical margin, which is paler (Fig. 7-A); lateral seta 1 ..... ***moesta***
Caudal lamellae with acute or subacute apices (Figs. 7-B,C); lateral setae 1-4 3

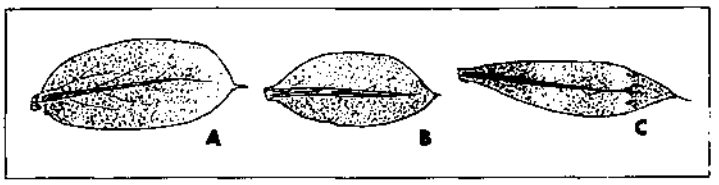

FIGURE 7. *Argia.* Lateral caudal lamella of: A - *A. moesta;* B - *A. apicalis;* C - *A. tibialis.*

3 (2) Lateral setae 2-4; lateral lamellae widest in the middle (Fig. 7-B) ..... ***apicalis***
Lateral seta 1; lateral lamellae widest beyond middle (Fig. 7-C) ..... ***tibialis***

KEY TO NYMPHS OF *NEUROCORDULIA*

1 Pyramidal horn on front of head ..... ***molesta***
No distinct horn on front of head 2

2 (1) Lateral spines on abdominal segment 9 greatly surpass tips of paraprocts; dorsal hooks blunt and erect ..... ***obsoleta***
Lateral spine on abdominal segment 9 barely reaching tips of paraprocts; dorsal hooks blunt and low, slanting to the rear ..... ***yamaskanensis***

KEY TO LARVAE OF *BRACHYCENTRUS*

1 Head entirely dark 2
Head with distinct light markings (Figs. 8-A,B) 3

2 (1) First abdominal sternum with 4 setae; metacoxal lobe surrounded by more than 30 setae ..... ***occidentalis***
First abdominal sternum with 2 setae; metacoxal lobe surrounded by about 11 setae .... ***americanus***

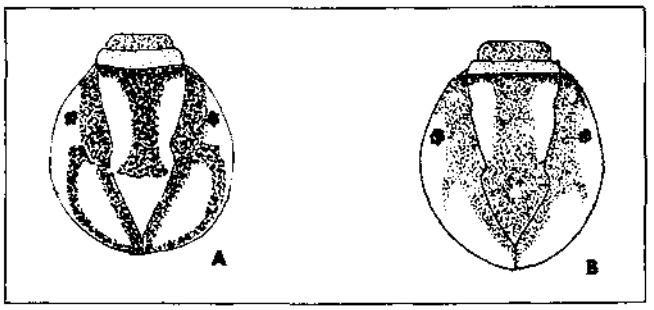

FIGURE 8. *Brachycentrus.* Dorsal view of head of: A - *B. lateralis;* B - *B. numerosus.*

3 (1) Head pale with dark stripes paralleling epicranial sutures, and a median dark stripe on frons (Fig. 8-A); pronotum with an anterior dark stripe and a dark stripe along transverse fold ..... ***lateralis***
Front of head mostly dark, with large pale marks laterally on frons (Fig. 8-B); pronotum without distinct transverse stripes ..... ***numerosus***

KEY TO LARVAE OF *MICRASEMA*

1 Head dark with pale stripes along frontal sutures and behind eyes; mesonotum with a single seta at SA-1 ..... ***kluane***
Head pale with darker muscle scars; mesonotum with a row of several setae at SA-1 2

2 (1) Case of sand grains; head with distinct, dark, rounded muscle scars on back of head, most nearly as large as eye; one or two pairs of pale spots usually on anterior of frontoclypeus (Fig. 9-A) ..... ***rusticum***

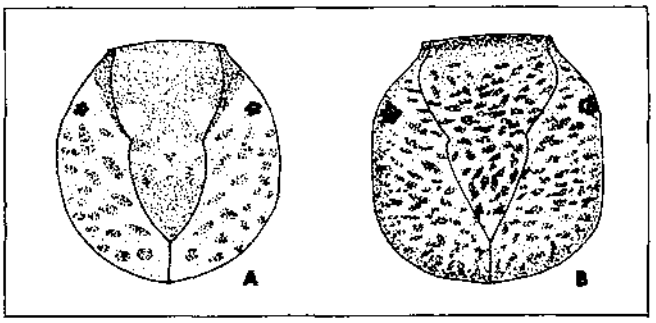

FIGURE 9. *Micrasema.* Dorsal view of head of: A - *M. rusticum;* B - *M. wataga.*

Case of fibers of vegetation; muscle scars on back of head highly variable in size and shape, frequently contiguous, and often indistinct; anterior of frontoclypeus without large pale spots (Fig. 9-B) ..... ***wataga***

KEY TO LARVAE OF *CHIMARRA*

1 Apex of frons with a pair of large, rounded lobes (Fig. 10-A) ..... ***obscura***
Apex of frons with smaller lobes, the left lobe not rounded 2

2 (1) Basal notch of right mandible very deep, with basal and apical side of notch subequal in length (Fig. 10-E); apex of frons with a small, rounded right lobe (Fig. 10-B) ..... ***socia***
Basal notch of right mandible with apical side much shorter than basal side (Figs. 10-F,G); right lobe on apex of frons larger (Figs. 10-C,D) 3

3 (2) Basal notch of right mandible shallow and forming a right angle (Fig. 10-F); apex of frons usually with a broad notch to the left of the right lobe (Fig. 10-C) ..... ***aterrima***
Basal notch of right mandible acute and deep (Fig. 10-G); apex of frons usually with an indistinct or narrow notch to the left of the right lobe (Fig. 10-D) ..... ***feria***

KEY TO LARVAE OF *HYDROPSYCHE*

1 Frontoclypeus with 2 large upturned teeth on anterior margin (Figs. 11-A,B) 2
Frontoclypeus without 2 large upturned teeth on anterior margin 3

2 (1) Posterior of head yellow with only a very narrow dark line on stem of epicranial suture (Fig. 11-A); frontoclypeus with a V-shaped pale mark..... ***orris***
Posterior of head with a broad dark mark along stem of epicranial suture (Fig. 11-B); frontoclypeus with 2 large anterolateral pale spots ..... ***bidens***

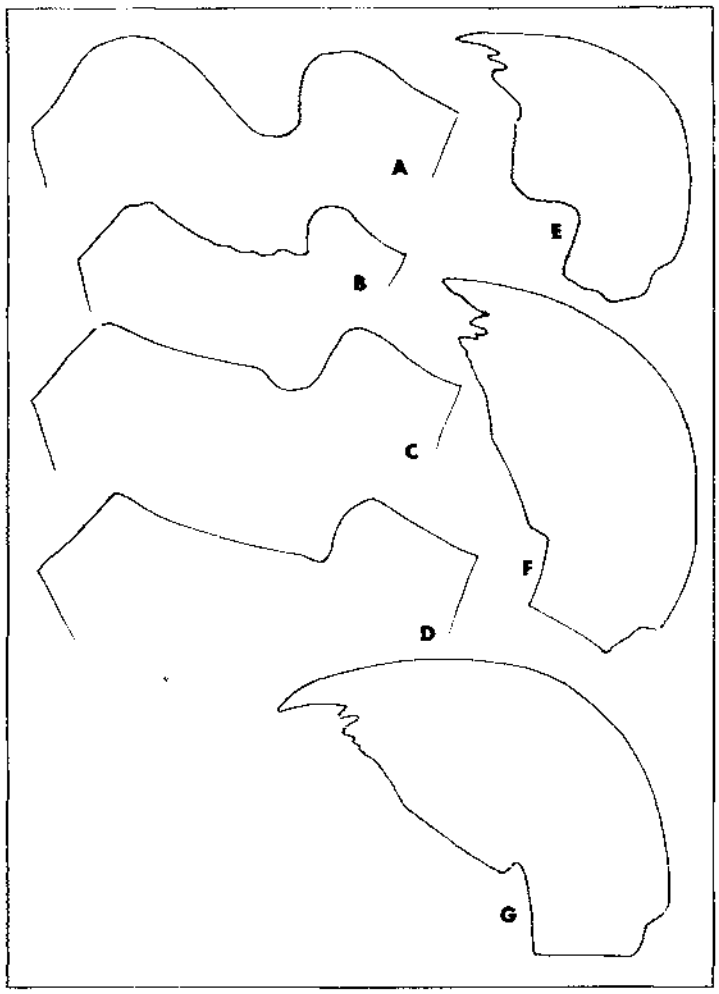

FIGURE 10. *Chimarra*. Apex of frons of: A - *C. obscura;* B - *C. socia;* C - *C. aterrima;* D - *C. feria.* Dorsal view of right mandible of: E - *C. socia;* F - *C. aterrima;* G - *C. feria.*

3 (1) Anterior edge of frontoclypeus produced (Fig. 11-C); head pattern as in Fig. 11-C ..... *phalerata*
Anterior edge of frontoclypeus straight or broadly rounded; head pattern otherwise 4

4 (3) Large spine-like setae, similar to those on sclerotized area of abdominal sternum 9, on venter of anal legs 5
Large spine-like setae absent from venter of anal legs 9

5 (4) Frontoclypeus mostly dark, with at most small pale spots (Figs. 11-D,E) 6
Frontoclypeus with large pale areas anteriorly (Figs. 11-F,G) 8

6 (5) Frontoclypeus brown with 2 pairs of distinct pale spots (Fig. 11-D); numerous dark, bristle-like setae on posterior of frontoclypeus ..... *valanis*
Frontoclypeus mottled brown with indistinct pale spots (Fig. 11-E) or light brown without spots; pale, spine-like setae on frontoclypeus 7

7 (6) Frontoclypeus mottled brown with indistinct pale spots (Fig. 11-E); posterolateral and ventral areas of head pale, with rows of muscle scars behind eyes; pronotum with distinct dark areas at base of pale spinelike setae and with large pale muscle scars laterally ..... *scalaris*
Frontoclypeus not mottled with indistinct pale spots; entire head light brown without distinct muscle scars, but with a patch of darker setae between eye and occiput; pronotum light brown without lateral muscle scars and insertions of pale spine-like setae only slightly darker than background ..... probably *placoda*

8 (5) Head with a brown patch on stem of epicranial suture; dark areas of frontoclypeus contiguous (Fig. 11-F); spine-like setae on anal legs same size as those on 9th abdominal sternum .... *aerata*

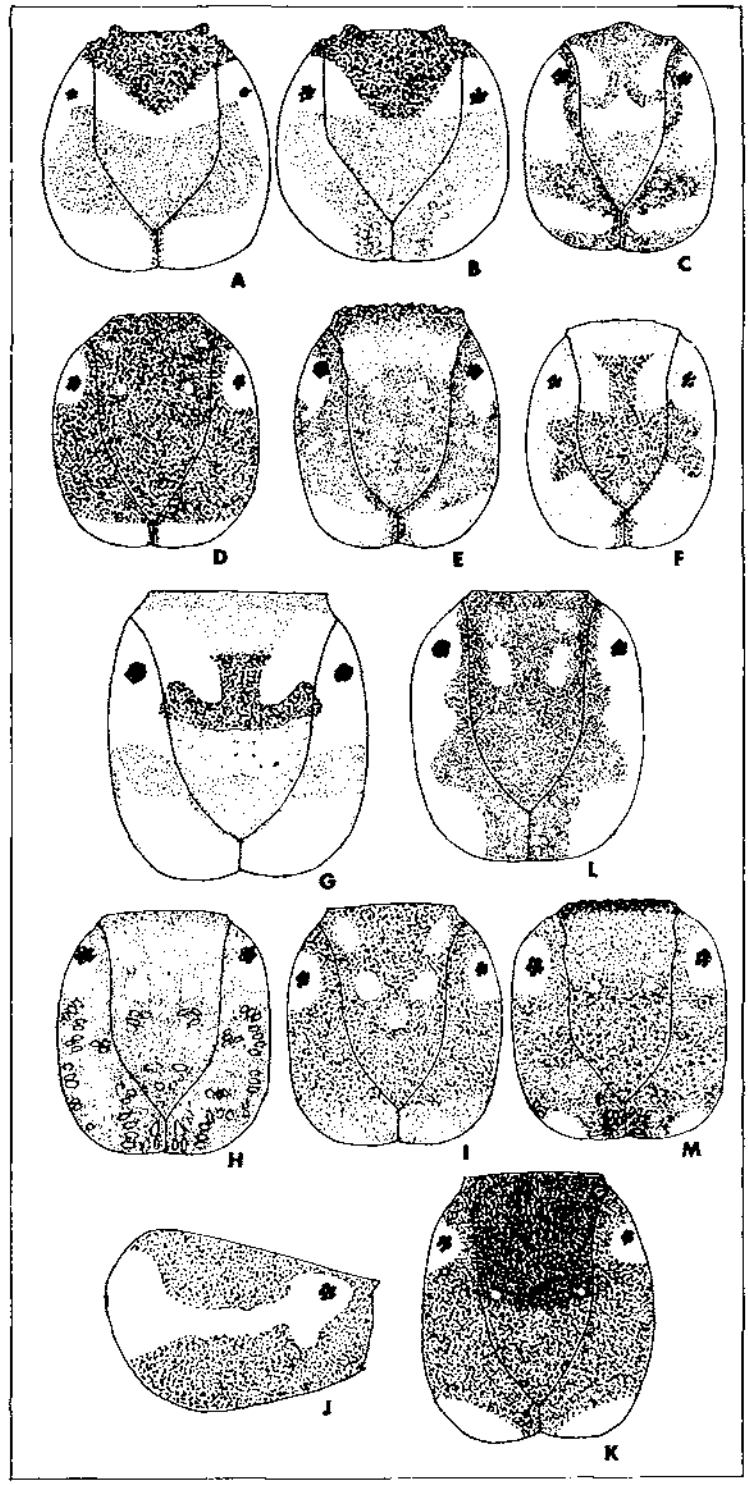

FIGURE 11. *Hydropsyche.* Dorsal view of head of: A - *H. orris;* B - *H. bidens;* C - *H. phalerata;* D - *H. valanis;* E - *H. scalaris;* F - *H. aerata;* G - *H. frisoni;* H - *H. cuanis;* I - *H. dicantha;* J - Lateral view of head of *H. leonardi.* Dorsal view of head of: K - *H. leonardi;* L - *H. simulans;* M - *H. arinale.*

Back of head entirely pale; dark area of frontoclypeus separated by pale areas (Fig. 11-G); spine-like setae on anal legs much smaller and weaker than those on 9th abdominal sternum ..... *frisoni*

9 (4) Frontoclypeus with a distinct elevated mound at extreme posterior;entire head usually dark brown except for a pale area around each eye, but occasionally, especially in early instars, larvae have pale spots on frontoclypeus ..... ***betteni***

Frontoclypeus without an elevated mound; head with distinct pale markings in addition to those around eyes..... 10

10 (9) Appressed thin setae sparse on all abdominal segments; scale hairs thin and club-shaped; top and sides of head a mottled reddish-brown with numerous pale muscle scars posteriorly and 2 or 4 indistinct pale spots on anterior of frontoclypeus (Fig. 11-H) ..... ***cuanis***

Appressed thin setae abundant on all abdominal segments; scale hairs broad, except in *dicantha;* head not marked as above..... 11

11 (10) Head mostly dark brown with 4 distinct pale spots and often a less distinct posterior spot on frontoclypeus (Fig. 11-I); many dark bristle-like setae on posterior of frontoclypeus..... ***dicantha***

Head not brown with 4 distinct pale spots on frontoclypeus; dark bristle-like setae absent from posterior of frontoclypeus, except in *leonardi*.... 12

12 (11) Top and sides of head dark brown with pale areas around eyes and at occiput that are usually connected to form a "duckling-shaped" mark (Fig. 11-J); often with a pair of small pale spots on frontoclypeus and inconspicuous muscle scars near back of head (Fig. 11-K) 13

Top and sides of head mottled reddish brown with conspicuous yellow muscle scars at back of head and behind eyes; pale areas may be present on frontoclypeus..... 14

13 (12) Frontoclypeus with dark bristle-like setae posteriorly; venter of head without quadrate yellow spots ..... *leonardi*

Frontoclypeus without dark bristle-like setae; venter of head with 2 quadrate yellow spots along gular suture ..... ***hageni***

14 (12) Scale hairs as abundant as thin appressed hairs on dorsum of middle abdominal segments; head with extensive posterolateral pale areas; frontoclypeus with 2 large anterolateral pale areas, that may be obscure (Fig. 11-L) ..... ***simulans***

Scale hairs sparse on abdominal segments 1-4, more numerous on segments 7-8; head a mottled reddish brown without extensive posterolateral pale areas; frontoclypeus with at most 2 pairs of small pale spots (Fig. 11-M) ..... ***arinale***

KEY TO LARVAE OF *SYMPHITOPSYCHE*

1 Frontoclypeus with several light spots forming a checkerboard pattern, the spots sometimes coalescing to form extensive light areas ..... *bifida* group

Light spots on frontoclypeus less than 4 or lacking 2

2 (1) Frontoclypeus with a central yellow spot, and occasionally with spots anterior and/or posterior to the central spot; in dark-headed individuals, there is a pale spot on the gular suture ..... ***slossonae***

Spots on frontoclypeus, if present, are at anterolateral margins; gular suture darkly pigmented 3

3 (2) Head usually dark brown with a "W"-shaped dark marking ventrally, the arms of the "W" wide and not reaching anterior of head (Fig. 12-A); prominent seta at anterolateral margin of pronotum very long, about 4 times as long as adjacent setae ..... ***riola***

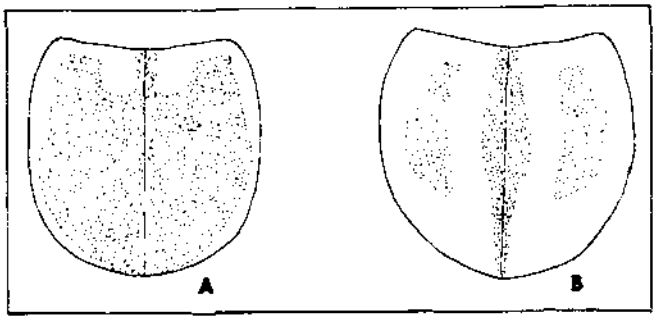

FIGURE 12. *Symphitopsyche.* Ventral view of head of: A - *S. riola;* B - *S. sparna.*

Head usually pale red-brown with dark mark on gular suture separated from lateral dark marks (Fig. 12-B); prominent seta at anterolateral margin of pronotum short, only about twice as long as adjacent setae ..... ***sparna***

KEY TO ADULTS OF *DUBIRAPHIA*

1 Large, length of elytra 2.1-2.4 mm..... ***bivittata***

Smaller, length of elytra 1.9 mm or less 2

2 (1) Elytra usually with four pale marks; if marks fuse to form two stripes, the stripes cover only the 3rd interval at basal third; elytra 1.6-1.9 mm long ..... ***quadrinotata***

Elytra with two pale stripes; if stripes are broken into four spots, elytra are less than 1.5 mm long.. 3

3 (2) Elytra less than 1.5 mm long; stripes narrow and sometimes obscure at basal third, conspicuously widened near middle to include 3rd to 6th intervals, and usually contrasting sharply with dark background ..... ***minima***

Elytra usually 1.6 mm long or longer; stripes nearly constant in width, only slightly wider near middle, and usually not contrasting sharply with dark background ..... ***vittata***

KEY TO ADULTS OF *OPTIOSERVUS*

1 Small, less than 2.2 mm long; yellow stripes on each elytron and a third stripe along elytral suture ..... ***trivittatus***

Large, more than 2.7 mm long; yellow stripes on each elytron, but without a stripe along elytral suture ..... ***fastiditus***

KEY TO KNOWN ADULTS OF *STENELMIS*

1 Last tarsal segment distinctly longer than the four preceding combined, the last segment suddenly

widened beyond the middle (Fig. 13-A) ; legs elongate 6

Last tarsal segment never distinctly longer than preceding segments combined, the last segment not suddenly widened beyond middle (Fig. 13-B) ; legs normal 2

2 (1) Orange stripe entirely on inside of elevated sixth interval; third interval sharply elevated at base ..... *cremata*

Orange stripe extending outside elevated sixth interval and covering basolateral corner of elytra 3

3 (2) Lower margin of last tarsal segment with a conspicuous angular process (Fig. 13-C) ; usually more than 3.2 mm long 4

Lower margin of last tarsal segment without a conspicuous angular process (Fig. 13-D) ; usually less than 3.2 mm long 5

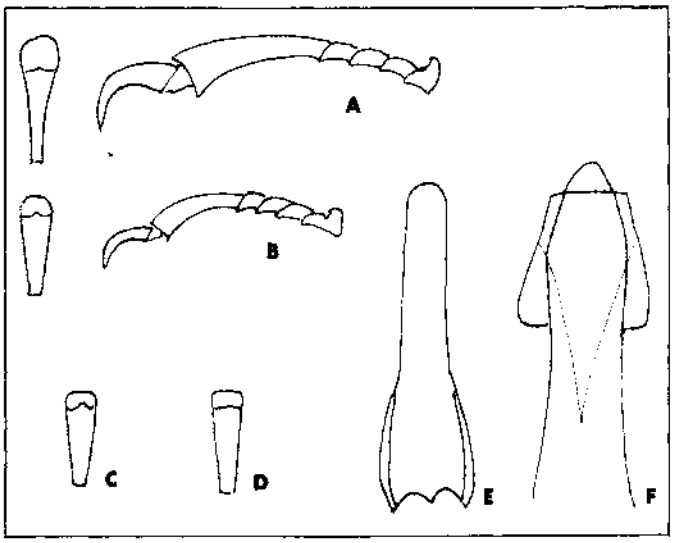

FIGURE 13. *Stenelmis.* Last tarsal segment (ventral) and tarsus (lateral) of: A - *S. decorata;* F - *S. crenata.* Last tarsal segment (ventral) of: C - *S. sandersoni;* D - *S. bicarinata.* Aedeagus of: E - *S. decorata;* F - *S. vittipennis.*

4 (3) Apical abdominal emargination equal to width of last tarsal segment; tibiae yellowish only at base ..... *concinna*

Apical emargination very inconspicuous and much less than width of last tarsal segment; tibiae and apices of femora yellowish ..... *sandersoni*

5 (3) Basal tubercle of pronotum elongate and carinate ..... *mera*

Basal tubercle just perceptibly elongate and never carinate ..... *bicarinata*

6 (1) Antennae or palpi, or both, dark brown or black ...7

Antennae and palpi yellowish 8

7 (6) Length 2.7 mm or longer..... *quadrimaculata*

Length less than 2.7 mm..... *musgravei*

8 (6) Length less than 3.0 mm; aedeagus lacks a lateral process (Fig. 13-E) ..... *decorata*

Length more than 3.0 mm; aedeagus with a prominent lateral process (Fig. 13-F) ..... *vittipennis*

KEY TO LARVAE OF *EUSIMULIUM*

1 Throat cleft square or nearly so (Fig. 14-A) 2

Throat cleft rounded anteriorly (Fig. 14-B) 4

2 (1) Dorsal head spots on a pale background 3

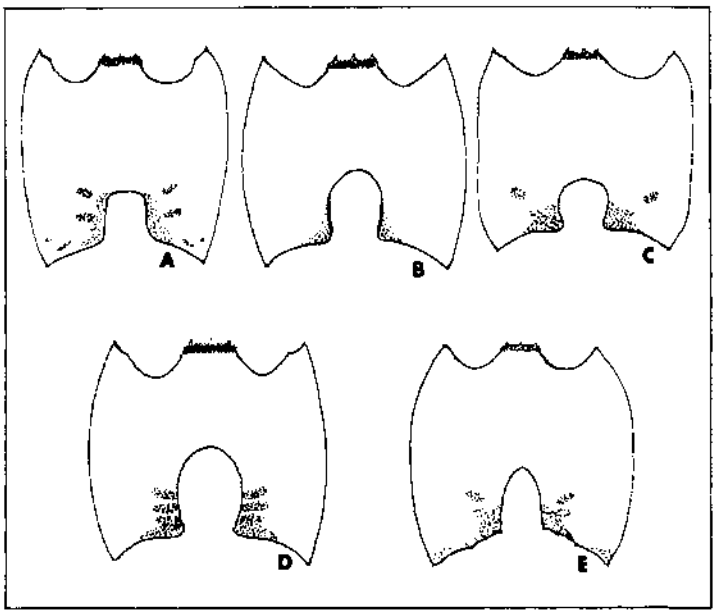

FIGURE 14. *Eusimulium.* Ventral view of head of: A - *E. aurium;* B - *E. croxtoni;* C - *E. latipes;* D - *E. gouldingi;* E - *E. pugetense.*

Dorsal head spots on a dark background ..... *euryadminiculum*

3 (2) Median tooth of mentum equal to or shorter than longest lateral tooth; anterolateral head spots distinctly separated; anal gill with compound lobes ..... *excisum*

Median tooth of mentum longer than lateral teeth; anal gill with 3 simple lobes; anterolateral head spots almost touching each other ..... *aurium*

4 (1) Pigmented area anteroventral to eye large 5

Pigmented area anteroventral to eye very small or absent 6

5 (4) Dorsal background pigment of head extended forward beyond bases of antennae as a dark, median stripe; throat cleft extending one-third distance to mental plate (Fig. 14-B) ..... *croxtoni*

Dorsal background pigment of head extended forward only to anterior head spot; throat cleft extending only one-fourth distance to mental plate (Fig. 14-C) ..... *latipes*

6 (4) Throat cleft large, rounded, bulbous (Fig. 14-D) ; small species maturing in summer..... *gouldingi*

Throat cleft small, widest at base (Fig. 14-E) ; large species maturing in early spring ..... *pugetense*

KEY TO LARVAE OF *SIMULIUM*

1 Throat cleft a small quadrangular emargination, extending only about one-fourth distance to apex of mental plate; anal gills with 3 simple lobes ..... *vittatum*

Throat cleft rounded or apically pointed, extending at least half way to apex of mental plate; anal gills with compound lobes 2

2 (1) Spots on head capsule light on a darker background 3

Spots on head capsule dark or obscure 5

3 (2) Infuscation around head spots narrow, not extending beyond inner edge of anterolateral spots; large, mature larvae 8-10 mm long ..... *decorum*

Infuscation around head spots extending beyond inner edge of anterolateral spots; mature larvae 6-7 mm long 4

4 (3) Lateral plate of proleg lightly sclerotized, barely visible; anterolateral head spots not enclosed by dark area ..... *verecundum*

Lateral plate of proleg heavily sclerotized, conspicuous; anterolateral head spots enclosed by dark area ..... *venustum*

5 (2) Throat cleft bulbous and extending about half way to apex of mental plate (Fig. 15-A) 6

Throat cleft parallel-sided or elongate, not distinctly bulbous (Figs. 15-B,C) 7

6 (5) Pupal histoblast of mature larva with 10 filaments ..... *jenningsi*

Pupal histoblast of mature larva with 12 filaments ..... *luggeri*

7 (5) Large median tooth of mentum extending far beyond lateral teeth; throat cleft short, parallel-sided basally, and pointed anteriorly (Fig. 15-B); mature larvae 10-11 mm ..... *pictipes*

Large median tooth of mentum not much longer than large lateral teeth; throat cleft variable; length of mature larvae less than 9 mm 8

8 (7) Throat cleft very long and slightly bulbous, extending almost to mental plate (Fig. 15-C) ..... *rugglesi*

Throat cleft not as above, pointed anteriorly (Figs. 15-D,E) 9

9 (8) Throat cleft with a distinct, narrow, apical extension extending almost to base of mentum (Fig. 15-D) ..... *corbis*

Throat cleft pointed anteriorly, but without a distinct apical extension (Fig. 15-E); head spots often obscure ..... *tuberosum*

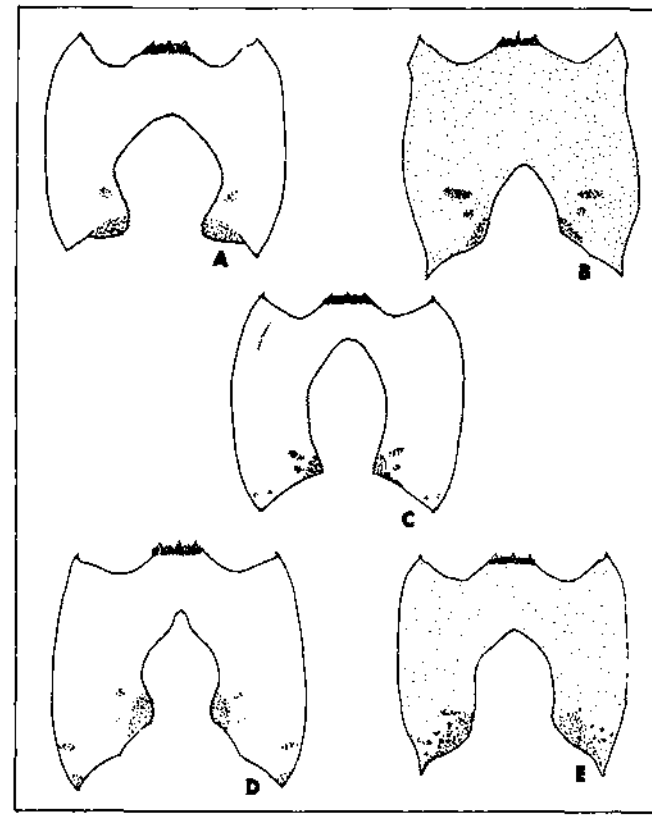

FIGURE 15. *Simulium*. Ventral view of head of: A - *S. jenningsi;* B - *S. pictipes;* C - *S. rugglesi;* D - *S. corbis;* E - *S. tuberosum.*

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TOLERANCE VALUES

PLECOPTERA

Capniidae: all *Allocapnia* 1*, all *Paracapnia* 1
Chloroperlidae: *Hastaperla brevis* 0, all *Alloperla* 0
Leuctridae: all *Leuctra* 0
Nemouridae: all *Amphinemura* 0, all *Nemoura* 0, all *Prostoia* 0, all *Shipsa* 0, all *Soyedina* 0
Perlidae: all *Acroneuria* 0, *Attaneuria ruralis* 1, *Neoperla clymene* 1, *Paragnetina media* 1, *Perlesta placida* 2, *Perlinella drymo* 1, *Perlinella ephyre* 0, *Phasganophora capitata* 0
Perlodidae: all *Isogenoides* 0, *Isoperla bilineata* 0, *I. clio* 0, *I. cotta* 0, *I. dicala* 0, *I. frisoni* 0, *I. lata* 0, *I. marlynia* 0, *I. nana* 2, *I. richardsoni* 2, *I. signata* 1, *I. slossonae* 0, *I. transmarina* 0
Pteronarcyidae: *Pteronarcys* spp. 1**
Taeniopterygidae: *Oemopteryx glacialis* 0, *Strophopteryx fasciata* 1, *Taeniopteryx* spp. 1

EPHEMEROPTERA

Baetidae: *Baetis brunneicolor* 2, *B. frondalis* 2, *B. flavistriga* 2, *B. intercalaris* 3, *B. longipalpus* 3, *B. macdunnoughi* 2, *B. propinquus* 2, *B. pygmaeus* 2, *B. vagans* 1, *Callibaetis* spp. 3, *Centroptilum* spp. 1, *Cloeon alamance* 1, *Cloeon* spp. 2, *Heterocloeon curiosum* 1, *Pseudocloeon carolina* 1, *P. dubium* 2, *P. parvulum* 2, *P. punctiventris* 2
Baetiscidae: all *Baetisca* 2
Caenidae: *Brachycercus* spp. 2, *Caenis* spp. 3
Ephemerellidae: *Attenella attenuata* 1, all *Danella* 1, all *Drunella* 0, *Ephemerella aurivillii* 0, *E. dorothea* 0, *E. excrucians* 1, *E. invaria* 1, *E. needhami* 1, *E. subvaria* 1, *Ephemerella* sp. A 1, *Eurylophella bicolor* 1, *E. funeralis* 0, *E. lutulenta* 3, *E. temporalis* 4, *Serratella deficiens* 1, *S. sordida* 0
Ephemeridae: *Ephemera simulans* 1, all *Hexagenia* 3
Heptageniidae: *Arthroplea bipunctata* 2, *Epeorus vitrea* 0, *Heptagenia diabasia* 3, *H. hebe* 1, *H. lucidipennis* 1, *H. pulla* 0, all *Rhithrogena* 0, *Stenacron interpunctatum* 3, *Stenonema exiguum* 3, *S. femoratum* 3, *S. integrum* 1, *S. mediopunctatum* 2, *S. modestum* 0, *S. pulchellum* 1, *S. terminatum* 2, *S. vicarium* 1
Leptophlebiidae: *Choroterpes basalis* 1, *Habrophlebiodes americana* 2, *Leptophlebia* spp. 2, *Paraleptophlebia* spp. 1
Polymitarcidae: *Ephoron leukon* 1
Potamanthidae: all *Potamanthus* 2
Metretopodidae: all *Siphloplecton* 1
Oligoneuriidae: *Isonychia* spp. 2
Siphlonuridae: *Ameletus* spp. 0, *Siphlonurus* spp. 2
Tricorythidae: *Tricorythodes* spp. 2

ODONATA

Aeshnidae: all *Aeshna* 3, *Anax junius* 3, *Basiaeschna janata* 2, *Boyeria vinosa* 1
Calopterygidae: all *Calopteryx* 2, *Hetaerina americana* 2
Coenagrionidae: *Amphiagrion saucium* 3, *Argia apicalis* 3, *A. moesta* 2, *A. tibialis* 2, *Chromagrion conditum* 3, *Coenagrion resolutum* 3, all *Enallagma* 3, *Ischnura verticalis* 4
Cordulegastridae: *Cordulegaster maculatum* 1
Corduliidae: all *Epitheca* 2, *Neurocordulia molesta* 2, *N. obsoleta* 1, *N. yamaskanensis* 1, *Somatochlora* spp. 0
Gomphidae: all *Gomphurus* 1, all *Gomphus* 2, *Hagenius brevistylus* 1, *Hylogomphus brevis* 1, *Ophiogomphus* spp. 1, *Stylogomphus albistylus* 0
Lestidae: all *Lestes* 3
Macromiidae: *Didymops transversa* 2, *Macromia illinoiensis* 1

TRICHOPTERA

Brachycentridae: *Brachycentrus americanus* 0, *B. lateralis* 0, *B. numerosus* 1, *B. occidentalis* 1, *Micrasema kluane* 0, *M. rusticum* 1, *M. wataga* 1
Glossosomatidae: *Agapetus* spp. 1, *Glossosoma* spp. 1, *Protoptila* spp. 1
Helicopsychidae: *Helicopsyche borealis* 2
Hydropsychidae: *Cheumatopsyche* spp. 3, *Diplectrona modesta* 0, *Hydropsyche arinale* 3, *H. betteni* 3, *H. bidens* 2, *H. cuanis* 3, *H. dicantha* 2, *H. leonardi* 1, *H. orris* 2, *H. phalerata* 1, *H. placoda* 2, *H. scalaris* 2, *H. simulans* 3, *Macronema zebratum* 2, *Parapsyche apicalis* 0, *Potamyia flava* 2, *Symphitopsyche bifida* group 3, *S. riola* 2, *S. slossonae* 2, *S. sparna* 1
Hydroptilidae: *Agraylea* spp. 3, *Hydroptila* spp. 3, *Leucotrichia* spp. 3, *Neotrichia* spp. 3, *Ochrotrichia* spp. 3
Lepidostomatidae: all *Lepidostoma* 1
Leptoceridae: all *Ceraclea* 2, *Leptocerus americanus* 2, *Mystacides sepulchralis* 2, all *Nectopsyche* 2, all *Oecetis* 2, all *Setodes* 2, all *Triaenodes* 2
Limnephilidae: *Anabolia* spp. 2, *Asynarchus montanus* 2, *Goera stylata* 0, *Hesperophylax designatus* 1, *Hydatophylax argus* 1, *Ironoquia* spp. 2, *Limnephilus* spp. 2, *Nemotaulius hostilus* 2, *Neophylax* spp. 2, *Onocosmoecus quadrinotatus* 1, *Platycentropus* spp. 2, *Psychoglypha subborealis* 0, *Pycnopsyche* spp. 2
Molannidae: all *Molanna* 1
Odontoceridae: *Psilotreta indecisa* 0
Philopotamidae: *Chimarra aterrima* 2, *C. feria* 1, *C. obscura* 2, *C. socia* 0, *Dolophilodes distinctus* 0, *Wormaldia moestus* 0
Phryganeidae: *Agrypnia* spp. 2, *Oligostomis ocelligera* 1, *Phryganea* spp. 2, *Ptilostomis* spp. 2
Polycentropodidae: *Cyrnellus fraternus* 3, *Neureclipsis* spp. 4, *Nyctiophylax* spp. 1, *Phylocentropus placidus* 1, *Polycentropus* spp. 2
Psychomyiidae: *Lype diversa* 1, *Psychomyia flavida* 2
Rhyacophilidae: all *Rhyacophila* 0
Sericostomatidae: *Agarodes distinctum* 2

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* "all *Allocapnia*" indicates all known Wisconsin species have a value of 1.
** "*Pteronarcys* spp. 1" indicates that species cannot be identified and the genus has been assiged a value of 1.

MEGALOPTERA

Corydalidae: all *Chauliodes* 2, *Corydalis cornutus* 2, *Nigronia serricornis* 1
Sialidae: *Sialis* spp. 2

LEPIDOPTERA

Pyralidae: *Neocataclysta* spp. 1, *Nymphula* spp. 1, *Paraponyx* spp. 1, *Parargyractis* spp. 2

COLEOPTERA

Dryopidae: all *Helichus* 2
Elmidae: *Ancyronyx variegata* 2, *Dubiraphia bivittata* 2, *D. minima* 3, *D. quadrinotata* 3, *D. vittata* 3, *Dubiraphia* larvae 3, *Macronychus glabratus* 2, *Microcylloepus pusillus* 1, *Optioservus fastiditus* 2, *O. trivittatus* 1, *Optioservus* larvae 2, *Stenelmis bicarinata* 2, *S. crenata* 3, *S. decorata* 2, *S. musgravei* 3, *S. sandersoni* 2, *S. vittipennis* 2, *Stenelmis* larvae 3
Gyrinidae: *Dineutus* larvae 2, *Gyrinus* larvae 2 (Do not count adults)
Psephenidae: *Ectopria* spp. 2, *Psephenus herricki* 2
(Do not include adults or larvae of Dytiscidae, Haliplidae or Hydrophilidae)

DIPTERA

Athericidae: *Atherix variegata* 2
Blepharoceridae: *Blepharocera* spp. 0
Ceratopogonidae: *Atrichopogon* spp. 1, *Bezzia* spp. 3, *Culicoides* spp. 4, *Monohelea* spp. 3, *Palpomyia* spp. 3, *Probezzia* spp. 3
Chaoboridae: all *Chaoborus* 4
Chironomidae: *Ablabesmyia* spp. 3, *Acricotopus* spp. 4, *Brillia* spp. 3, *Cardiocladius* spp. 3, *Chaetocladius* spp. 3, *Chironomus* spp. 5, *Cladopelma* spp. 4, *Cladotanytarsus* spp. 3, *Clinotanypus* spp. 3, *Coelotanypus* spp. 2, *Cordites* spp. 2, *Corynoneura* spp. 2, *Cricotopus* spp. 4, *Cryptochironomus* spp. 4, *Cryptotendipes* spp. 3, *Demicryptochironomus* spp. 3, *Diamesa* spp. 2, *Dicrotendipes* spp. 4 *Diplocladius* spp. 4, *Einfeldia* spp. 5, *Endochironomus* spp. 3, *Epoicocladius* spp. 2, *Eukiefferiella* spp. 2, *Glyptotendipes* spp. 5, *Guttipelopia* spp. 3, *Harnischia* spp. 4, *Heterotrissocladius* spp. 2, *Hydrobaenus* spp. 2, *Kiefferulus* spp. 4, *Larsia* spp. 3, *Limnophyes* spp. 3, *Microchironomus* spp. 4, *Microcricotopus* spp. 3, *Micropsectra* spp. 3, *Microtendipes* spp. 3, *Nanocladius* spp. 1, *Natarsia* spp. 3, *Nilotanypus* spp. 3, *Odontomesa* spp. 2, *Orthocladius* spp. 3, *Pagastia* spp. 2, *Parachironomus* spp. 4, *Paracladopelma* spp. 3, *Paralauterborniella* spp. 3, *Parametriocnemus* spp. 3, *Paratanytarsus* spp. 3, *Paratendipes* spp. 2, *Pentaneura* spp. 2, *Phaenopsectra* spp. 4, *Polypedilum* spp. 3, *Potthastia* spp. 2, *Procladius* spp. 3, *Prodiamesa* spp. 2, *Psectrocladius* spp. 2, *Psectrotanypus* spp. 3, *Pseudochironomus* spp. 3, *Pseudorthocladius* sp. 2, *Rheocricotopus* spp. 3, *Rheotanytarsus* spp. 3, *Saetheria* spp. 2, *Smittia* spp. 4, *Stempellina* spp. 2, *Stempellinella* spp. 2, *Stenochironomus* spp. 2, *Stictochironomus* spp. 3, *Sympotthastia* spp. 2, *Tanypus* spp. 4, *Tanytarsus* spp. 3, *Thienemanniella* spp. 2, *Thienemannimyia* complex 3, *Xenochironomus* spp. 2, *Zalutschia* spp. 2, *Zavrelimyia* spp. 4
Dolichopodidae: all genera 2
Empididae: all genera 3
Ephydridae: all genera 3
Muscidae: all genera 2
Psychodidae: *Pericoma* spp. 5, *Psychoda* spp. 5
Ptychopteridae: *Ptychoptera* spp. 3
Simuliidae: *Cnephia dacotensis* 1, *Ectemnia taeniatifrons* 1, *Eusimulium aurium* 2, *E. croxtoni* 1, *E. euryadminiculum* 1, *E. johannseni* 1, *E. latipes* 2, all *Prosimulium* 1, *Simulium corbis* 0, *S. jenningsi* 2, *S. luggeri* 1, *S. tuberosum* 2, *S. venustum* 3, *S. verecundum* 3, *S. vittatum* 4, *Stegopterna mutata* 2
Syrphidae: *Chrysogaster* spp. 5, *Eristalis* spp. 5, *Helophilus* spp. 5
Tabanidae: *Chrysops* spp. 3, *Tabanus* spp. 3
Tipulidae: *Antocha* spp. 2, *Dicranota* spp. 2, *Erioptera* spp. 3, *Helius* spp. 3, *Hesperoconopa* spp. 1, *Hexatoma* spp. 3, *Limonia* spp. 2, *Limnophila* spp. 2, *Pedicia* spp. 2, *Pilaria* spp. 3, *Pseudolimnophila* spp. 1, *Tipula* spp. 2
(Do not include Culicidae, Dixidae, or Stratiomyidae)

AMPHIPODA

Gammaridae: *Crangonyx gracilis* 4, *Gammarus pseudolimneus* 2
Talitridae: *Hyallela azteca* 4

ISOPODA

Asellidae: *Asellus intermedius* 5

RECENT SYNONYMS

	New Name	Old Name
EPHEMEROPTERA	*Baetis flavistriga*	*Baetis levitans* or *Baetis phoebus*
	Baetis longipalpus	*Baetis propinquus*
	Baetis propinquus	*Baetis spinosus*
	Macdunnoa persimplex	*Stenonema persimplex*
	Stenonema femoratum	*Stenonema tripunctatum*
	Stenonema modestum	*Stenonema rubrum*
	Stenonema terminatum	*Stenonema bipunctatum*
	Stenonema vicarium	*Stenonema fuscum*
	Attenella attenuata	*Ephemerella attenuata*
	Danella	*Ephemerella* (in part)
	Drunella	*Ephemerella* (in part)
	Eurylophella	*Ephemerella* (in part)
	Serratella	*Ephemerella* (in part)
ODONATA	*Epitheca*	*Epicordulia* or *Tetragoneuria*
TRICHOPTERA	*Symphitopsyche*	*Hydropsyche* (in part)
DIPTERA	Athericidae	Rhagionidae
	Brundinella	*Psectrotanypus* (in part)
	Cladopelma	*Cryptocladopelma* (in part)
		Harnischia (in part)
	Hydrobaenus	*Trissocladius*
	Macropelopia	*Psectrotanypus*
	Microchironomus	*Cladopelma*
	Nanocladius	*Plecopteracoluthus*
	Prodiamesinae	Diamesinae (in part)
	Zalutschia	*Rheocricotopus* (in part)
	Ectemnia taeniatifrons	*Cnephia taeniatifrons*
	Stegopterna	*Cnephia* (in part)

CALCULATION AND EVALUATION OF BIOTIC INDEX VALUES

After all the necessary identifications have been completed, the number of arthropods in each species (or genus) is multipled by the tolerance value for the species (or genus), and the sum of these products is divided by the number of arthropods in the entire sample to obtain the biotic index for the stream

$$B.I. = \frac{\sum n_i n_a}{N}$$

Samples obtained between October and May give the most reliable values and can be evaluated according to Table 7. Accurate correction factors for values obtained from summer samples have not yet been worked out, but the results in Table 5 suggest that subtracting 0.6 from biotic index values obtained in July and August is not unreasonable. A smaller correction factor will be needed for June and September samples.

TABLE 7. Evaluation of water quality using biotic index values of samples collected between October and May.

Biotic Index	Water Quality	Degree of Organic Pollution
0.00 - 1.75	Excellent	No organic pollution
1.76 - 2.25	Very good	Possible slight organic pollution
2.26 - 2.75	Good	Some organic pollution
2.76 - 3.50	Fair	Significant organic pollution
3.51 - 4.25	Poor	Very significant organic pollution
4.26 - 5.00	Very Poor	Severe organic pollution

The occurrence of several *Caenis* spp., *Cheumatopsyche* spp., and *Symphitopsyche bifida* group, all of which have a tolerance value of 3, will produce abnormally high biotic index values for very clean streams. Calculation of a second biotic index after excluding these three genera is recommended, and if it is below 2.00 it should be used to evaluate the stream.

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About the Author

Dr. Hilsenhoff is Professor of Entomology, University of Wisconsin, Madison.

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